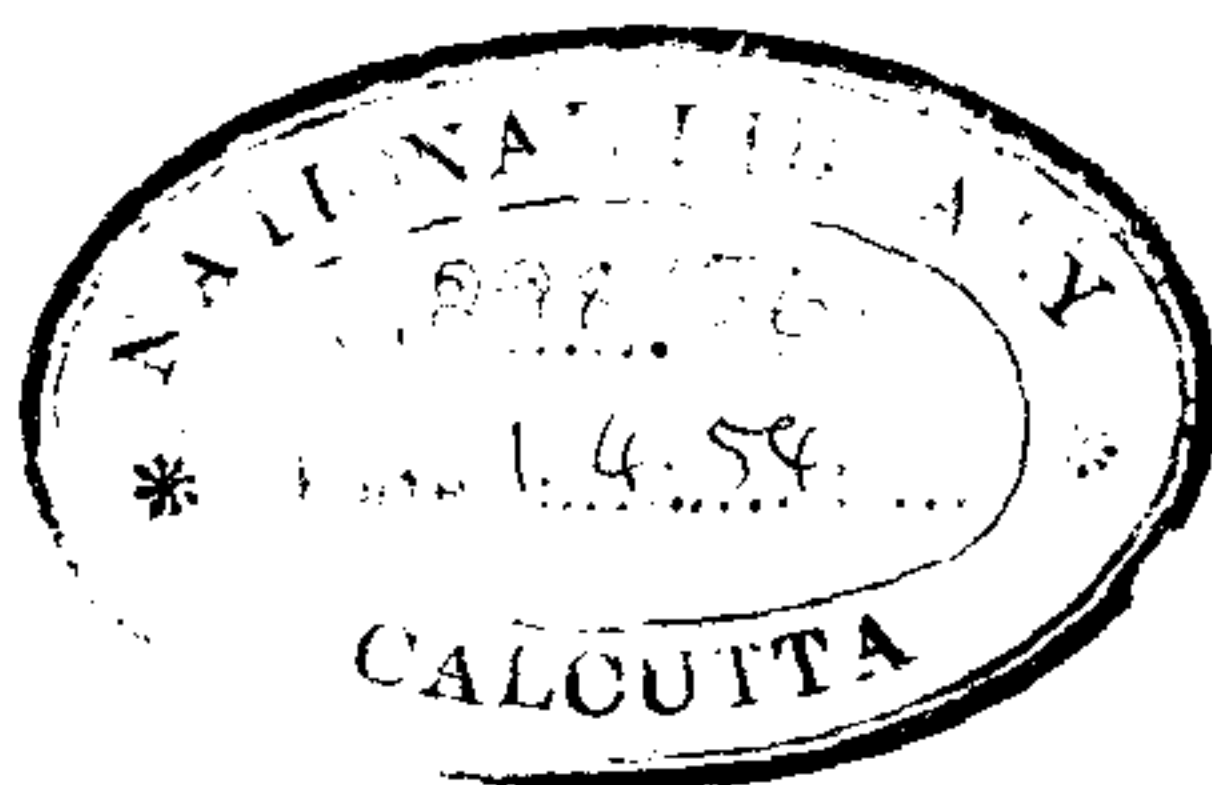


CYTOLOGICAL STUDIES IN BIGNONIACEAE

K. R. VENKATASUBBAN, M.Sc., L.T., Ph.D.,

Lecturer in Botany,

ANNAMALAI UNIVERSITY.



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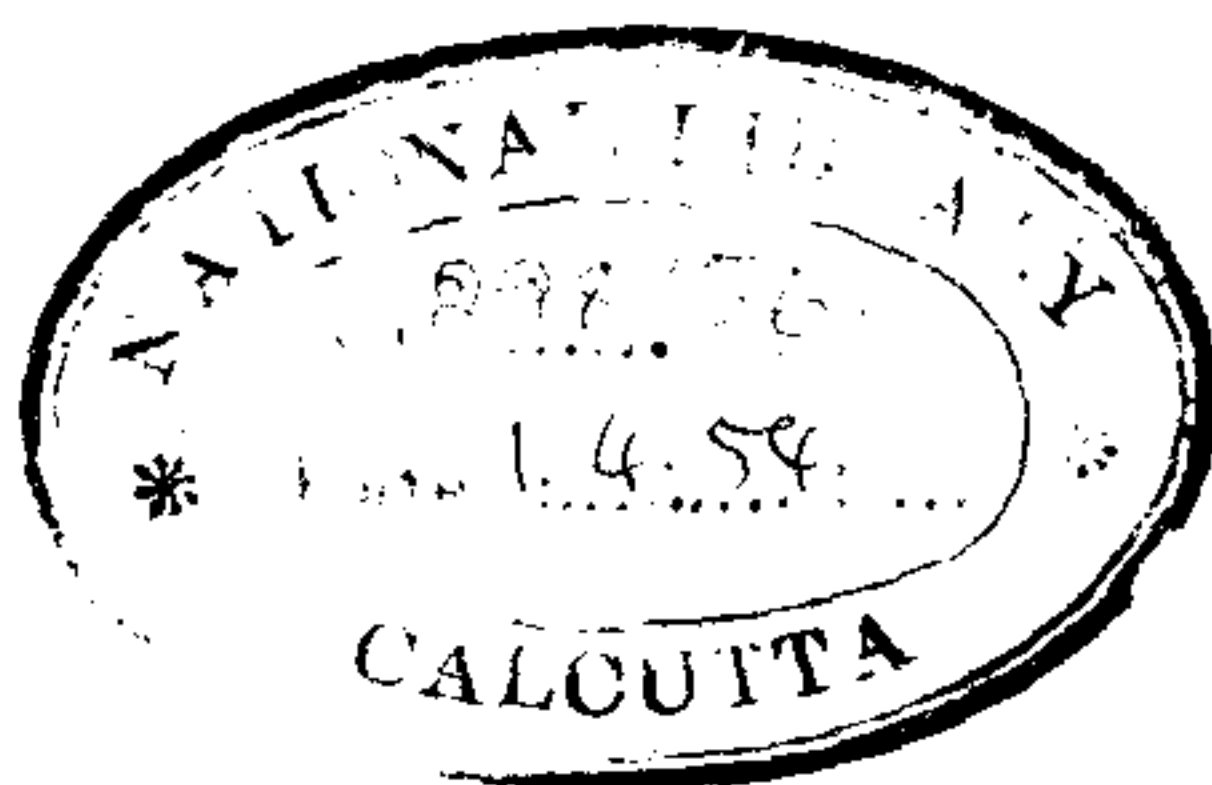


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FOREWORD

The significant part that chromosome numbers play in the field of taxonomy, genetics and cytology is now too well known to need any special emphasis. A knowledge of chromosome numbers of plant species is of the utmost importance not only to the cytologist and taxonomist, whose investigations may be regarded as academic, but also to the practical plant breeder. While some data pertaining to the chromosome numbers are available for plants of the temperate regions, very little information is on hand in regard to plants of the tropics in general and to those of the Indian tropics in particular. It is very desirable, therefore, that strenuous efforts are made systematically to fill up this lacuna in our knowledge.

The present work will, from this point of view, form a substantial contribution to the cytology of the predominantly tropical family Bignoniaceae. Except for a few stray chromosome counts, no cytological information of any kind has been recorded for any of the important members of this family. Chromosome numbers have been determined, mostly for the first time, for over 35 species spread over 16 genera. In

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itself this is a contribution of considerable importance. With the aid of chromosome numbers, so far available in this family including those determined in this investigation, an attempt has been made to formulate a tentative scheme of evolution of the different genera and the main lines along which speciation has progressed among the different genera have also been indicated. In formulating the scheme, the author has not relied on chromosome numbers alone. For, he has made a critical cytological study of some important genera like ~~Crescentia~~, Millingtonia, Tabebuia etc., and has utilised the cytological data gathered from their study for framing the scheme of evolution. For instance, he has recorded secondary association in a number of genera and by a critical examination of the associations, their frequency etc., has employed the phenomenon for purposes of elucidating problems of phylogeny. In this connection the author has taken great pains to collect almost all available information regarding plants in which secondary association has been recorded so far. This has been given as an appendix to Part II and should be of considerable use to those working in this field.

In *Kigelia pinnata*, of which the author has made a special cytological study, he has recorded the detailed behaviour of the nucleolus during meiosis. He has in that connection

VII

discussed at some length the problems connected with the nucleolus, its persistence and its possible significance. The nucleolus is coming to play an increasingly prominent role not only in the field of cytology but also in plant genetics.

On the whole, the three parts which formed the thesis for the author's doctorate will be an important contribution to our knowledge of the cytology of the hitherto little investigated *Bigoniaceae*. It is hoped that the data gathered and discussed will prove to be of some use and guidance to future workers in this family.

Botanical Laboratory,
Annamalai University,
February 8th, 1944.

} T. S. RAGHAVAN,
Professor of Botany.

Acknowledgements

I take great pleasure in acknowledging my indebtedness to Prof. T. S. Raghavan for his unfailing interest in the progress of the work and for much valuable suggestions and criticisms. My sincere thanks are due to him also for the unrestricted facilities extended to me to consult several references from his own collection of reprints.

In addition grateful acknowledgment is made to the Vice-Chancellor and Syndicate of the Annamalai University for all the help received from time to time and for the publication of the thesis.

MADRAS CHRISTIAN COLLEGE,

TAMBARAM.

15 Sept. 1945.

K. R. VENKATASUBBAN.

PART I

The Cytology of *Kigelia pinnata* DC with
Special Reference to Nucleolar Behaviour.

(With 1 plate and 38 text figures)

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PART I

THE CYTOLOGY OF *KIGELIA PINNATA* DC WITH SPECIAL REFERENCE TO NUCLEOLAR BEHAVIOUR*

I. INTRODUCTION

KIGELIA pinnata DC is a big-sized tree reaching a height of 20 to 50 feet and is found in large numbers in and around the city of Madras, mostly planted as avenue trees. It is a native of the Mozambique district in Africa, Bailey (1935). In certain parts of Africa, this tree is held sacred. According to Bailey (1925), the fruit of this species has some medicinal value. Hooker and Jackson (1893) mention only 5 species of *Kigelia*, while a dozen species are described by Bailey (1935). Most of these are confined to tropical Africa including the Island of Madagascar.

This species has not been studied so far either from the morphological or cytological point of view.

Materials for the present study were obtained from the Madras Agri-Horticultural

* Part I of thesis approved for the degree of Doctor of Philosophy of the Annamalai University.

gardens. Some implanted materials, chiefly for verificational purpose were got through Prof. Miss Kausalya of the Queen Mary's College, Madras, to whom my sincere thanks are due. For cytological preparations, young buds were dissected and the anthers were cut into small bits, the right stage of PMC development was determined from a preliminary examination in aceto-carmin. They were pre-fixed in Carnoy's fluid (6 parts of absolute alcohol, 3 parts of chloroform and 1 part of glacial acetic acid) for about 40 seconds, rinsed in water and finally fixed in Navashin's fluid. The Navashin's fluid was changed after one or two hours. After the lapse of 24 hours, the material was washed thoroughly in luke-warm water so as to remove all traces of chromic acid, as it otherwise leaves a stain. Dehydration was effected in the usual manner, with the different grades of alcohol starting with 5%. In the lower grades, the material was allowed to remain for only a couple of hours, while from 70% onwards, the time allowed was 4 hours or more. Chloroform was employed as the solvent for paraffin and after embedding, sections were cut at a thickness ranging from 12 to 14 microns. For staining, Newton's iodine-genetian-violet schedule was followed. For root tips also, the same procedure was adopted with regard to fixation and staining and sections were cut at a thickness of 16 microns.

II. CYTOLOGY

(a) *Somatic Chromosomes*: The chromosome number for this species is determined for the first time and it was found to be $2n=40$. The somatic chromosomes were found to be comparatively short and they have mostly median or sub-median attachment constriction. Though chromosome counts were made from different sectors of the root tips, there was no indication of any somatic polyploidy, either involving the entire complement or only a few. The presence or absence of somatic pairing could not be determined as most of the chromosomes were alike. Satellited chromosomes too, could not be made out, though several plates were examined and a careful search was made for these. The somatic number $2n=40$, was also confirmed by meiotic counts especially in the first metaphase, where n was found to be equal to 20. Prochromosomes were also noticed. The role and behaviour of these, which were found to be common in almost all the genera and species examined, are discussed at some length elsewhere. In *Oroxylum indicum*, where these bodies were very conspicuous, their complete cycle in mitosis has been traced and light thrown upon the exact nature of these bodies.

(b) *Meiosis*:—The pollen mother cells, at the onset of meiosis, are compact and have only angular walls. They do not round off and

separate, as is the case in many plants. At the resting stage, prophase and diakinesis, the nucleolus is very prominent. Some interesting quantitative observations which were made in regard to the relative volume of the nucleolus at different stages of meiosis, are given below :—

(In computing the diameter, camera lucida drawings of the nucleolus were made at the different stages and with the help of a planimeter the area was first found out and from which the diameter was calculated.)

	area in sq. cms., (average of 12 readings)	diam. in cms.,
Resting stage	3.76 sq. cms.	2.1880cms.
Diakinesis	1.23 „	1.2512 „
Metaphase and anaphase	} 0.68 sq. cm.	0.9304 cm.
Second telophase	0.79 „	1.0032cms.
Pollen tetrad	1.53 sq. cms.	1.3954 „

There is only one spherical conspicuous nucleolus, which in many cases was found to persist even beyond diakinesis. The determination of the organization of the nucleolus at the telophase was a matter of some difficulty; but usually 2 nucleoli are formed at this stage and these on fusion give rise to a single prominent nucleolus, seen in the resting and subsequent stages of development of the PMC.

On account of the fact that diakinesis is of extremely short duration, only very few PMCs

were found at this stage of meiosis. This coupled with the small size of the chromosomes, precluded the possibility of studying the bivalents in any detail at this stage. But this did not however preclude the observation of the important fact of the attachment of 2 bivalents to the nucleolus at mid-diakinesis. (Text fig. 2 and photomicrograph Plate I fig. 1) About a dozen pollen mother cells were examined and it was found, that the maximum number of bivalents attached to the nucleolus was only 2. The occurrence of 2 nucleoli at the first telophase and the occurrence of 2 bivalents attached to the nucleolus, would indicate that these attached bivalents represent the nucleolar chromosomes. In other words, in the monoploid complement, 2 satellited chromosomes must be presumed to be responsible for the organization of the nucleolus. It follows then, that in the diploid complement, there should be 4 SAT-chromosomes or chromosomes with secondary constrictions. But in none of the somatic plates examined, were satellited chromosomes or secondary constricted chromosomes ever found. It may be, that the small size of the chromosomes and the extremely delicate nature of the trabants prevented, these being distinguished optically. There is also the possibility of the SAT-chromosomes not being present, even though the nucleoli would appear to indicate and postulate their presence. This is discussed later down.

Such a direct relationship between the number of chromosomes attached to the nucleolus at prophase and the number of SAT-chromosomes, was found in a number of species of *Calceolaria*, Srinath (1939). A similar relationship between the number of attached bivalents to the nucleolus during diakinesis and the number of SAT-chromosomes, was found in *Gynandropsis pentaphylla*, Raghavan (1938) and again in *Crataeva religiosa*, Raghavan and Venkatasubban (1939).

In text fig. 2, though the full complement is not represented, it may be observed that, the bivalents are all of the rod-type. These, therefore, show only one terminal chiasma. This would suggest that the bivalents have only a weak affinity between the homologous chromosomes, presumably due to structural changes. This is supported by the occurrence of secondary association.

During DIAKINESIS, the nucleolus generally occupies a peripheral position in the nucleus and in just a few instances, it was even found outside the nucleus. It was thought that, this might be due to accident in cutting; but it is significant that in such cases, the nuclear membrane is rather faint and appears as though it has dissolved or about to dissolve. A very characteristic feature in the meiosis of this species, is the persistence of the nucleolus during meta-

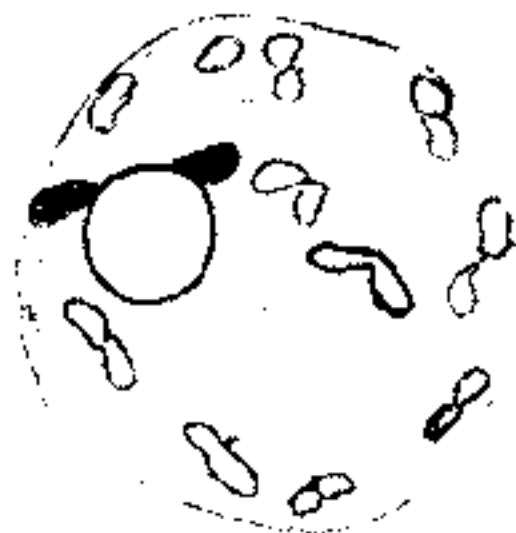
Explanation of text figures (1-14)

(All figs. have been drawn with a camera lucida at an approximate magnification of $\times 3600$ except figs. 12, 13 and 14 which were drawn at an approximate magnification of $\times 1800$. The drawings have been reduced to nearly $2/3$).

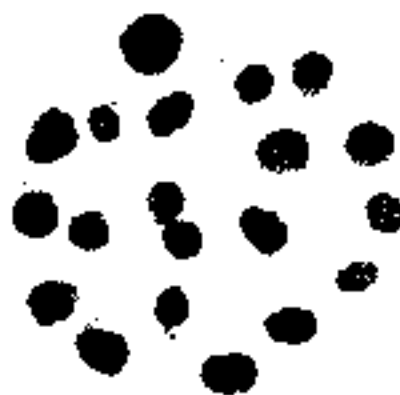
- Fig. 1. Somatic metaphase; $2n = 40$.
- „ 2. Diakinesis showing the attachment of 2 bivalents to the nucleolus and several rod bivalents.
- „ 3. First metaphase; $n = 20$. A few of these are found in groups of two.
- „ 4. Metaphase I (side view). Some of the bivalents are seen going towards the pole while the majority of them are still at the equator. The persistent nucleolus is also seen near the pole.
- „ 5. Anaphase I.
- „ 6. Anaphase I, somewhat spread out and appears as though it is a case fusion of two homotypic spindles.
- „ 7. Anaphase I, showing a few laggards which form a chain; same as photomicrograph, (Pl. 1, fig. 4).
- „ 8. Anaphase I where the chromosomes are distributed all over the spindle. Note that the spindle is very short.
- „ 9. Interphase; the two nuclei are seen quite close to each other.
- „ 10. First telophase; an elongated and bipolar spindle is seen.
- „ 11. First telophase, showing the crescent-shaped spindle and a few laggards near about the poles.
- „ 12. First telophase where the two nuclei are about to fuse; the spindle fibres are seen lying on one side without any reference to the nuclei. Same as photomicrograph (Plate 1, fig. 6).
- „ 13. Second anaphase; the spindles are seen orientated at right angles to each other.
- „ 14. Second telophase showing the fusion of the nuclei, cell-plate and a persistent nucleolus.



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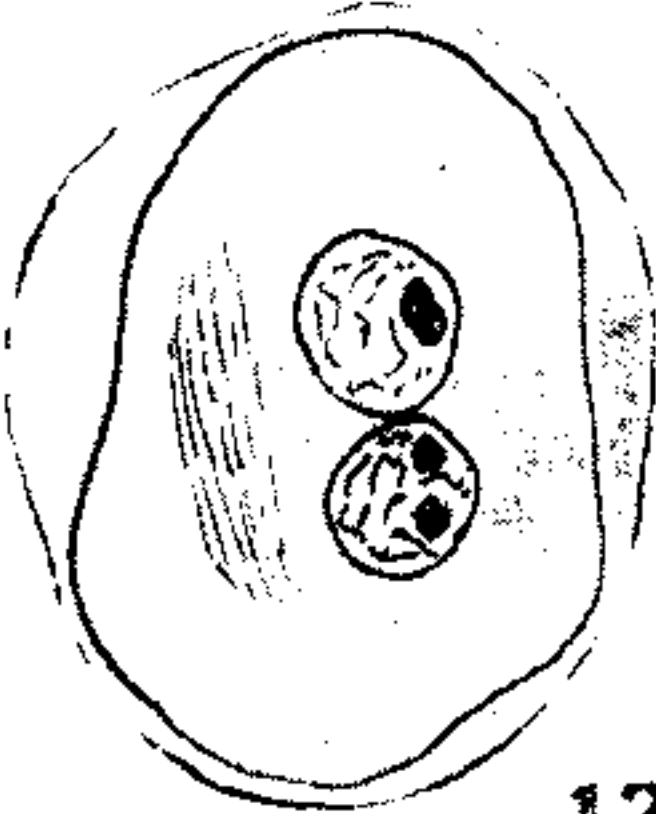
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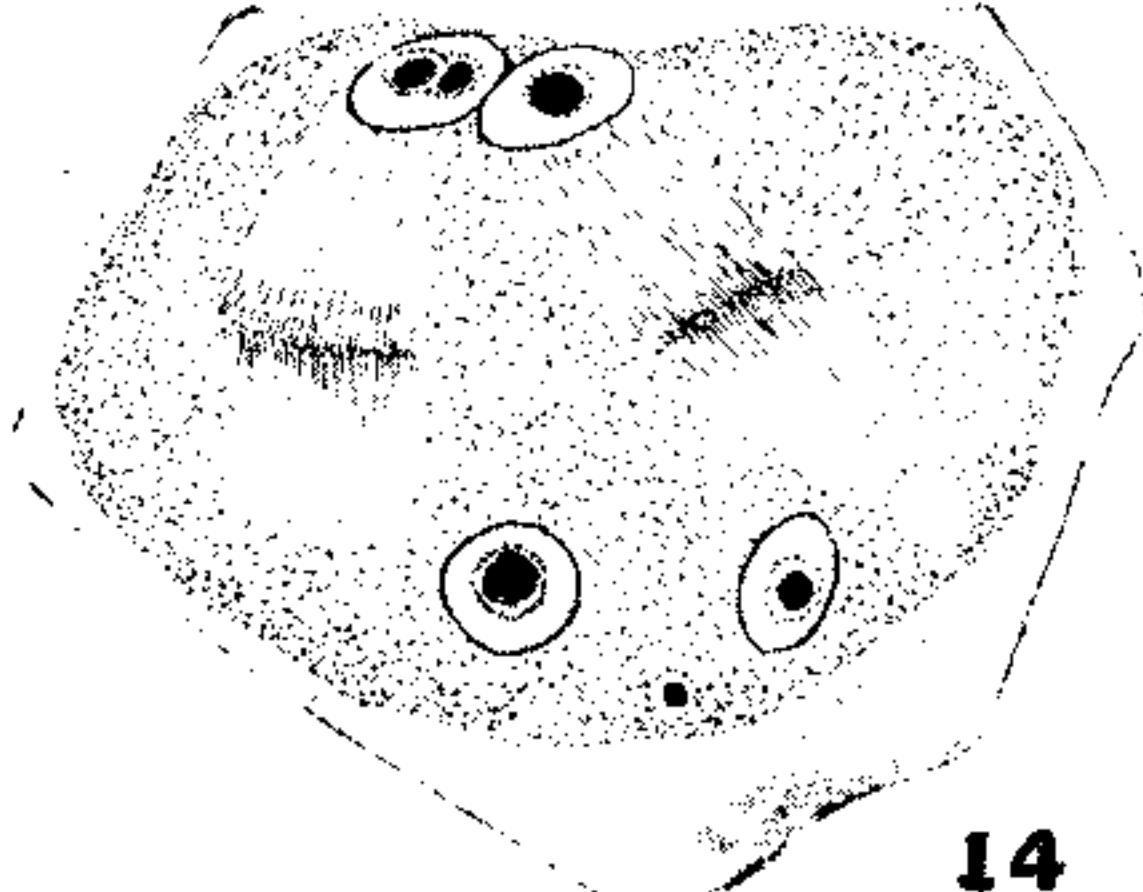
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phase and the above observation would suggest the manner in which the nucleolus comes to persist.

At METAPHASE I, 20 bivalents are seen. They are of different sizes (text fig. 3 and Photomicrograph Plate I fig. 2). In addition to these 20 bivalents, varying number of spherical bodies (1 to 4), very much bigger than the chromosomes were also met with. The exact nature of these spherical bodies and their behaviour are treated further down in this paper.

The FIRST ANAPHASE proceeds in the usual manner in the majority of the PMCs. However, there were found several instances, indicating a tendency towards non-disjunction. In fig. (14), while most of the bivalents are still at the equator, a few have already commenced their journey to the poles. The dumbbell shaped appearance of these, shows that these are bivalents reaching the pole as such. This would naturally result in unequal numbers of chromosomes in the poles and consequently in the tetrads. In text fig. 7, which represents the first anaphase, 7 chromosomes are found to lag and these form a chain (photomicrograph Pl. I fig. 4). In text fig. 8, the chromosomes are scattered all over the spindle. Text figures 5 and 6, represent anaphase I of a more regular character, as compared to figures 7 and 8. In text fig. 5, the chromosomes at the poles, are not spread out

and consequently, the diameter at the equator is only about half of what is seen in text fig. (6). In text fig. (6), the chromosomes at each pole have spread out, simulating the fusion of the two homotypic spindles. However, since each pole contains only about 20 chromosomes, it is merely a case of spreading out of the chromosomes and not due to any fusion or tendency to that effect. Text figure (11) shows the first telophase. Even at this stage, 4 chromosomes at one pole and a solitary one at the other are seen. Evidently these were lagging and when the telophasic nuclei are about to be organized, these laggards have moved to the poles. It cannot be said definitely as to whether these will be included or not, in the two daughter nuclei, which have been organized already. In the same figure, it can also be seen that the spindle is curved, presenting a crescent-shaped appearance. Similar crescent-shaped spindles were seen in appreciable numbers in the F_1 plants, in artificially raised interspecific hybrids of *Papaver bracteata* $\times$ *P. lateritium* by Yasui (1941). He regards such spindles as mere artifact caused by violent fixation. Fig. 10 represents the first telophase. The two telophasic nuclei are fairly apart and a membrane has been formed around each nucleus. It is noteworthy, that the spindle fibres, are clearly seen at this stage. The chromosomes are equally spaced out and show the characteristic constricted appearance. It is

at this stage, that the nucleoli are organized. The first telophase is succeeded by interphase or interkinesis. There is not much of a difference between the first telophase and interphase nuclei. In both the stages, the chromosomes are uniformly distributed as at diakinesis and they show the characteristic X-shaped configuration. However, in these preparations, it was found that during the first telophase, the spindle is quite visible, while in the interphase, it disappears. Further, in the interphase, there is a sort of reticulate appearance, (text fig. 9) similar to what is often discerned at the early prophase stage. It may also be pointed out, that during the early telophase stage, the chromosomes are quite nearer to one another and it is only during late telophase and interphase, that these become equidistant from one another. Such a uniform disposition of the chromosomes during interkinesis was first noted by Gates (1909) in *Oenothera*. He explained the clumped appearance of the chromosomes at the early telophase and their uniform spacing during the interphase, as due to mutual attraction and repulsion of the chromosomes. No cross wall has been noticed between the interphasic nuclei.

The interphase leads on to the 2nd pro-metaphase, prior to metaphase II. Second pro-metaphase was not observed in any of the PMC, probably due to its very short duration. Second

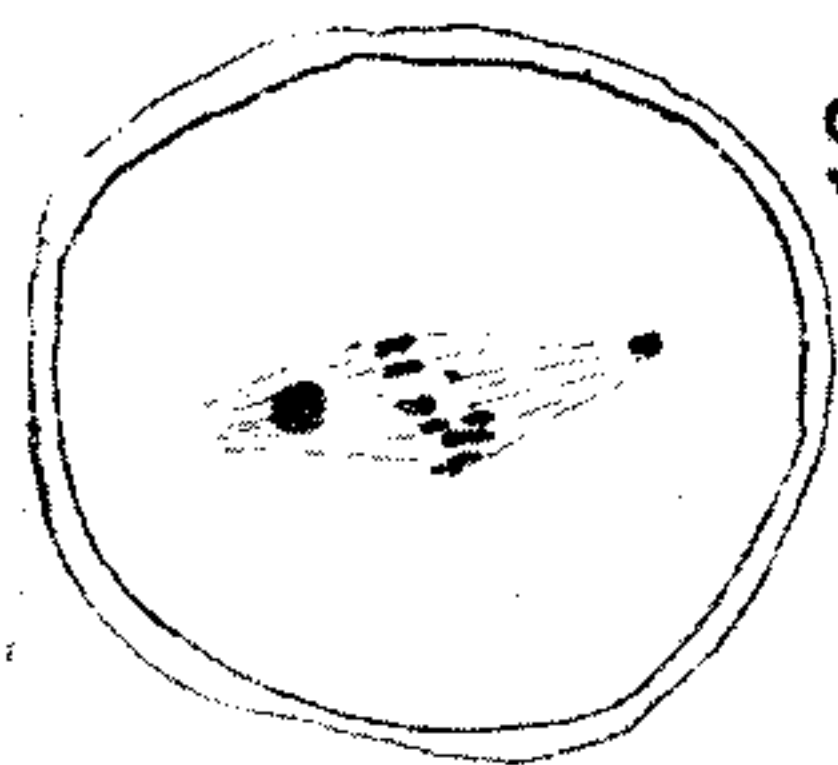
Metaphase plates were observed in a number of the pollen mother cells, but the chromosome number could not be determined, as most of these presented only a side view. The second metaphase leads on to the second anaphase, where the spindles may lie either at right angles or parallel to each other, (text figs. 13, 26 and 27). Tetrads are formed by the process of furrowing, with slight modifications, an account of which is given separately. Quite a large number of the tetrads formed, however, degenerate, leaving only a few healthy grains.

(c) *Persistent Nucleolus ; Its Behaviour :*
In a number of the PMCs., besides the 20 bivalents, there were found as a rule 1, 2 or 4 bodies. These were quite spherical and much bigger than the bivalents. These spherical bodies were found to be of the nature of nucleoli. These lie a little apart from the bivalents during Metaphase I, (text fig. 15 and photomicrograph Pl. I fig. 3). At first sight, these were mistaken for bivalents, but this was ruled out, as there are already found 20 bivalents excluding these bodies and that the presence of such big bivalents would involve, the presence of two or more very big chromosomes in the somatic metaphase plate. However, as can be seen from text fig. (1), the somatic chromosomes are pretty small without much disparity in size. On the other hand, its resemblance to the nucleolus is

Explanation of text figures (15-22)

(All figures have been drawn at a magnification of $\times 1800$, unless otherwise stated, and have been reduced to $\frac{3}{5}$ in reproduction.)

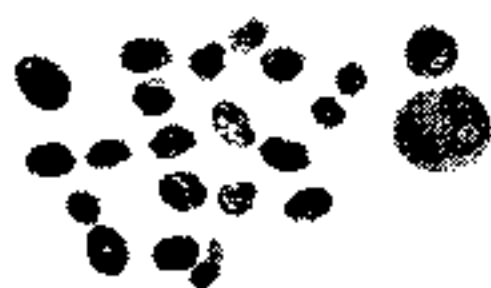
- Fig. 15. Metaphase I in which 20 bivalents and one persistent nucleolus are seen $\times 3600$. Same as Plate 1, fig. 3.
- „ 16. Metaphase I showing two persistent nucleoli one big and the other small; $\times 3600$.
- „ 17. MI with 4 persistent nucleoli, two big and two small.
- „ 18. Two unequal persistent nucleoli migrating to opposite poles during MI; same as photomicrograph: Pl. 1, fig. 9.
- „ 19. MI. Two persistent nucleoli are seen near one of the poles and one is in the act of division.
- „ 20. MI, showing one persistent nucleolus. Note that the bivalents are still at the equator.
- „ 21. Early telophase 1. Two nucleoli are seen, one at the equator and the other in the vicinity of the pole.
- „ 22. Telophase 1 with one persistent nucleolus at the equator.



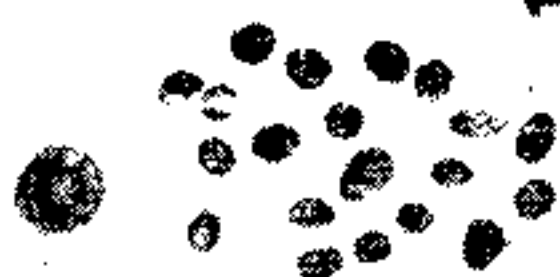
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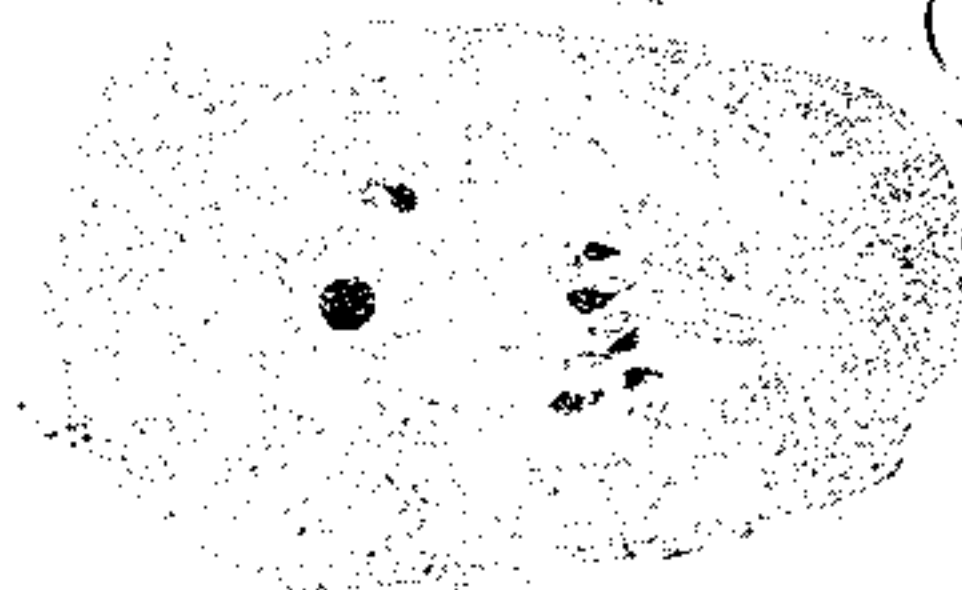
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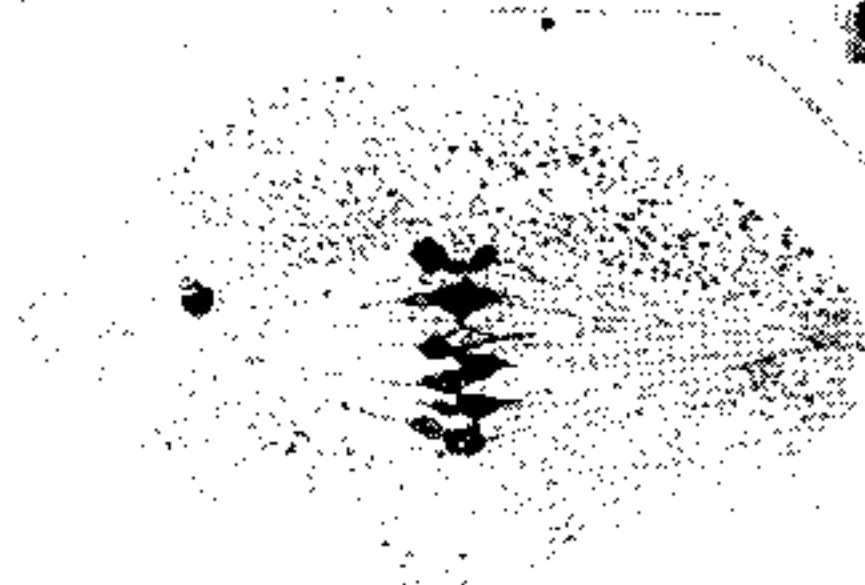
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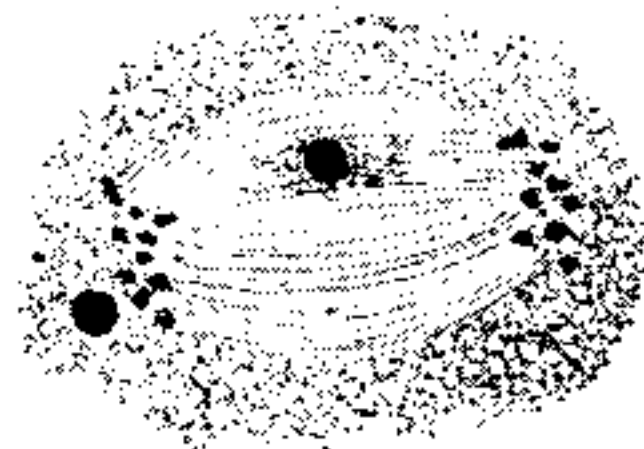
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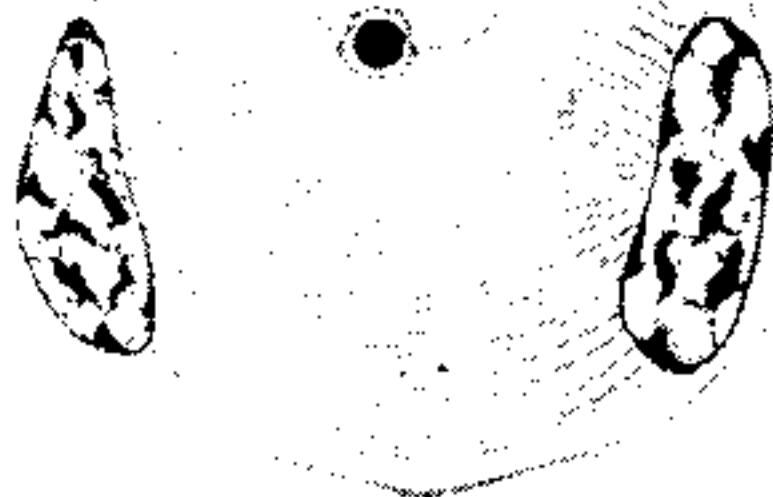
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pretty clear, both on account of its big size, rounded nature and also from the fact, that it takes up the stain to the same extent as the nucleolus. It is also stained by light green. From a number of other considerations also, which will be discussed further down, the conclusion was reached, that these spherical bodies which are seen in most of the PMCs. are nucleoli.

The persistent nucleolus is quite conspicuous by its size, especially when it lies by the side of the bivalents. Around this body is a halo, but there is no indication of the presence of any membrane. In most cases, the persistent nucleolus does not divide. At the time, when the bivalents are assembled at the equator during first metaphase, the persistent nucleolus migrates to the vicinity of the pole. The persistent nucleolus is not seen in all the pollen mother cells. In one and the same locus, some have it, while others do not show the same. The nucleolus persists in the first metaphase, (fig. 15), first telophase (figs 22, 23 & 24), second metaphase (fig. 27), 2nd anaphase (fig. 26) and 2nd telophase (figs 28 and 14).

In the majority of the cases, the persistent nucleolus does not divide, but remains single throughout the meiotic cycle. In such cases, the nucleolus migrates towards one of

the poles much earlier than the chromosomes, (figs. 4 & 20). After approaching the pole, it gets extruded into the surrounding cytoplasm and disappears (fig. 25). The nucleolus may sometimes instead of moving towards the pole, remain, at the equator as in figures 22 and 23. It remains in that position up to the telophase (photomicrograph Pl. I fig. 7). The nucleolus may also take a lateral course, as seen in figure 24.

Occasionally, the nucleolus divides into two unequal bodies during metaphase, as is seen in figure 16. During metaphase, when the bivalents are still at the equator, these two bodies are observed to migrate towards opposite poles, as in text figure 18 and photomicrograph Pl. I fig. 9, or the two bodies may migrate to one and the same pole, where one of these may undergo a further division as seen in figure 19. It may be that, while one of these bodies migrates towards the pole, the other may remain stationary at the equator as seen in text fig. 21.

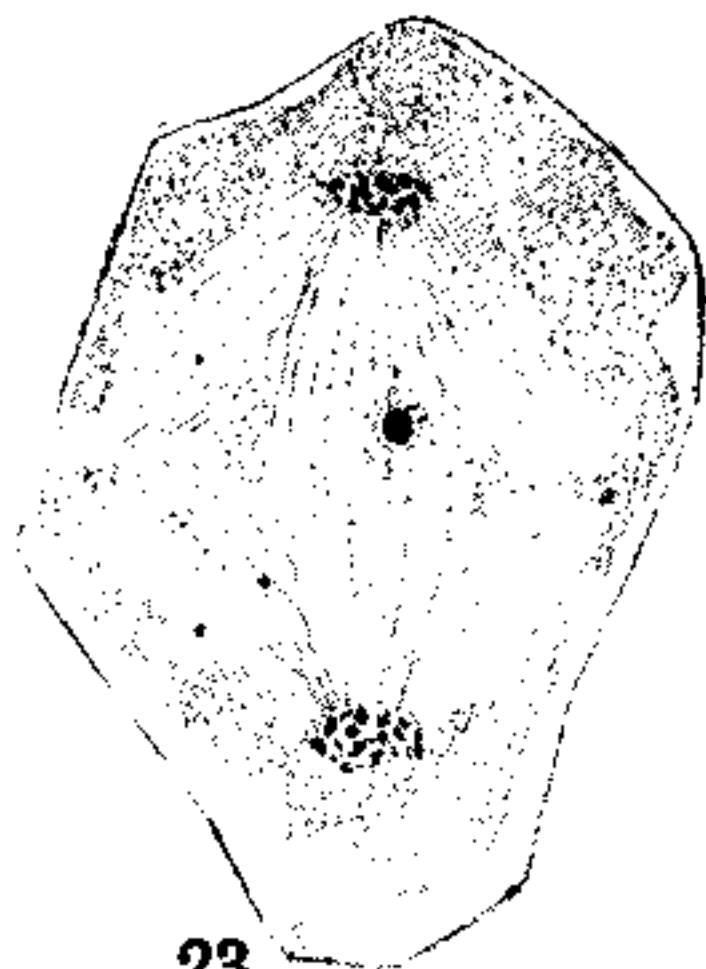
During metaphase, I, the nucleolus may undergo two repeated divisions, resulting in 4 bodies of unequal size as seen in text figure 17. The further behaviour of these bodies could not however be followed.

During the second metaphase, either both the plates or only one, may show the persistent

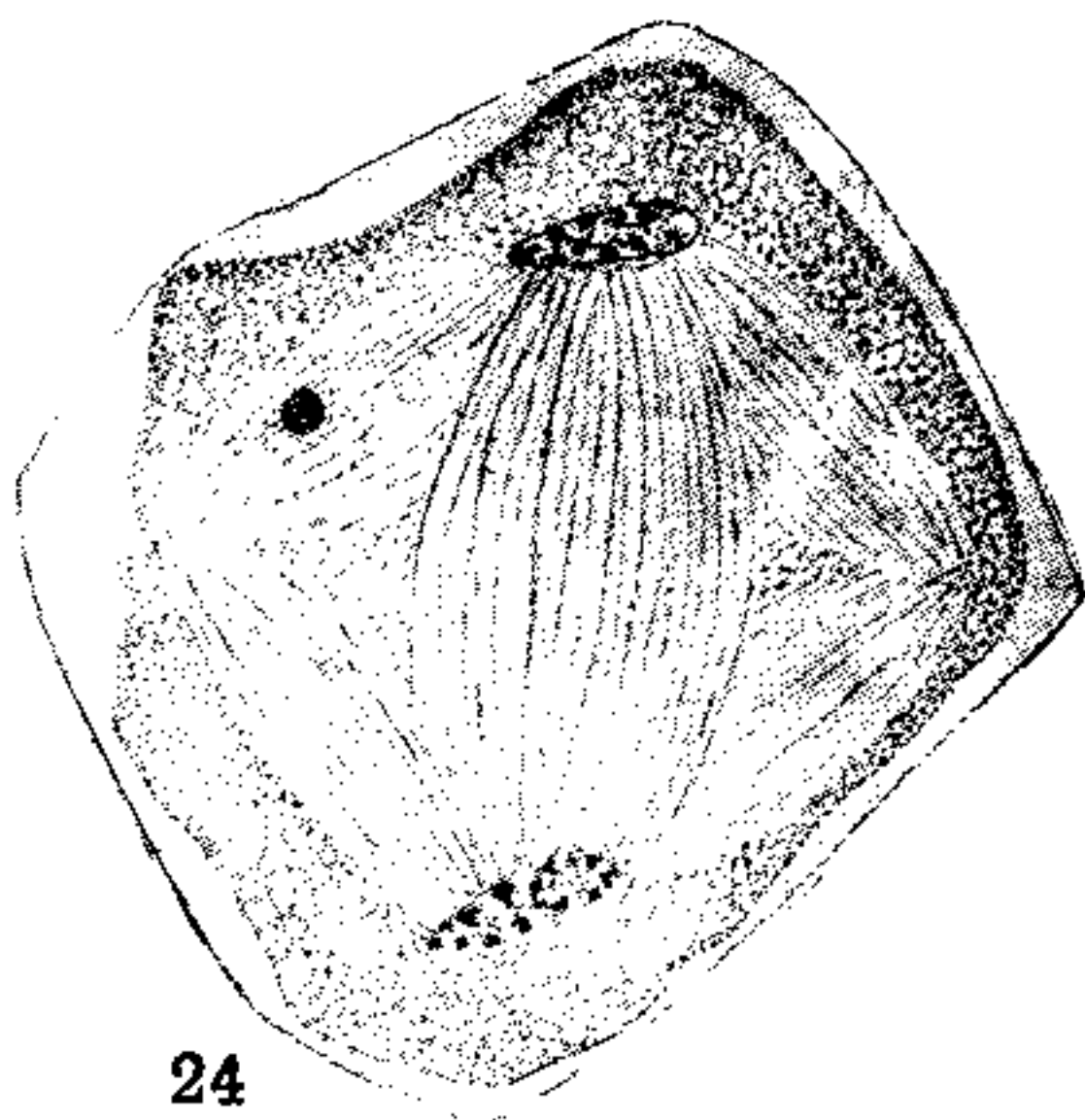
Explanation of text figures 23-28

(All figures have been drawn at a magnification of $\text{Ca} \times 1800$, unless otherwise stated, and have been reduced to $\frac{3}{5}$ in reproduction.)

- Fig. 23. First telophase with multipolar spindle and a persistent nucleolus lying at the equator in the axial spindle.
- „ 24. First telophase; the single persistent nucleolus is seen in one of the supernumerary spindles.
- „ 25. First telophase showing the supernumerary spindles and the persistent nucleolus lying in the cytoplasm very near the wall; same as photomicrograph. Pl. 1, fig. 10.
- „ 26. Second anaphase. In each plate a persistent nucleolus is seen lying at the equator.
- „ 27. Second metaphase. A single persistent nucleolus is seen in only one of the plates.
- „ 28. Second telophase, showing six spindles and a persistent nucleolus lying in the course of one of these.



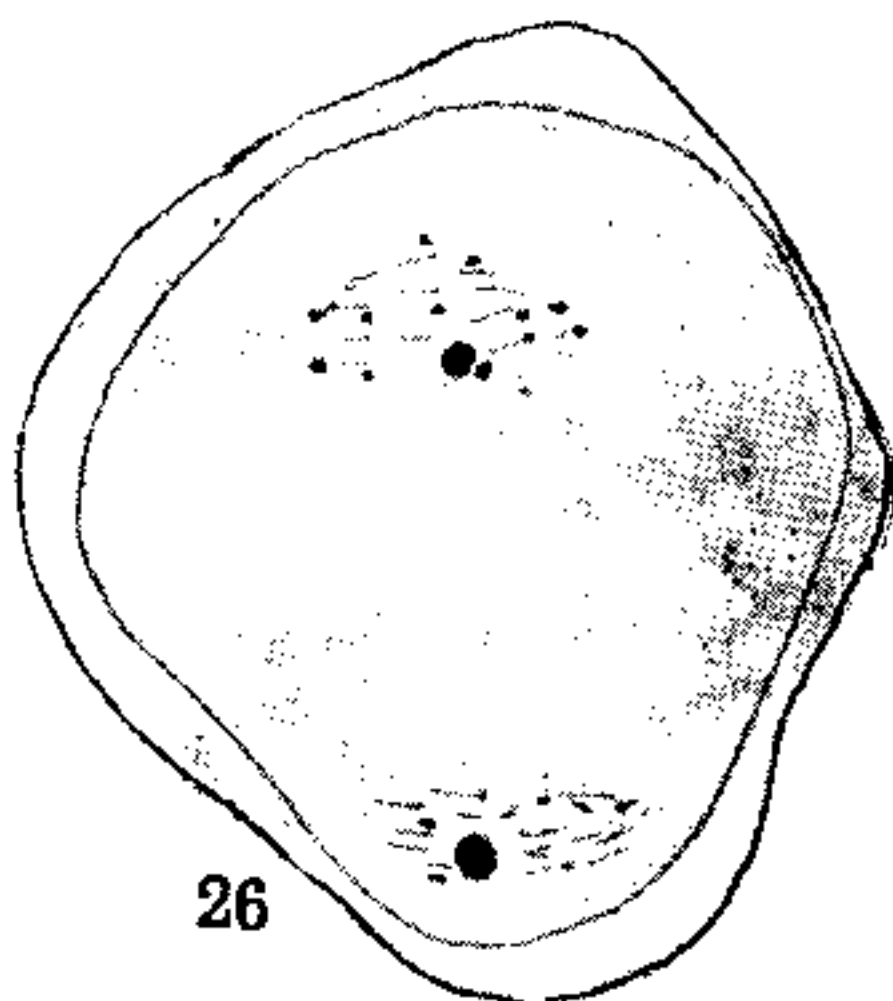
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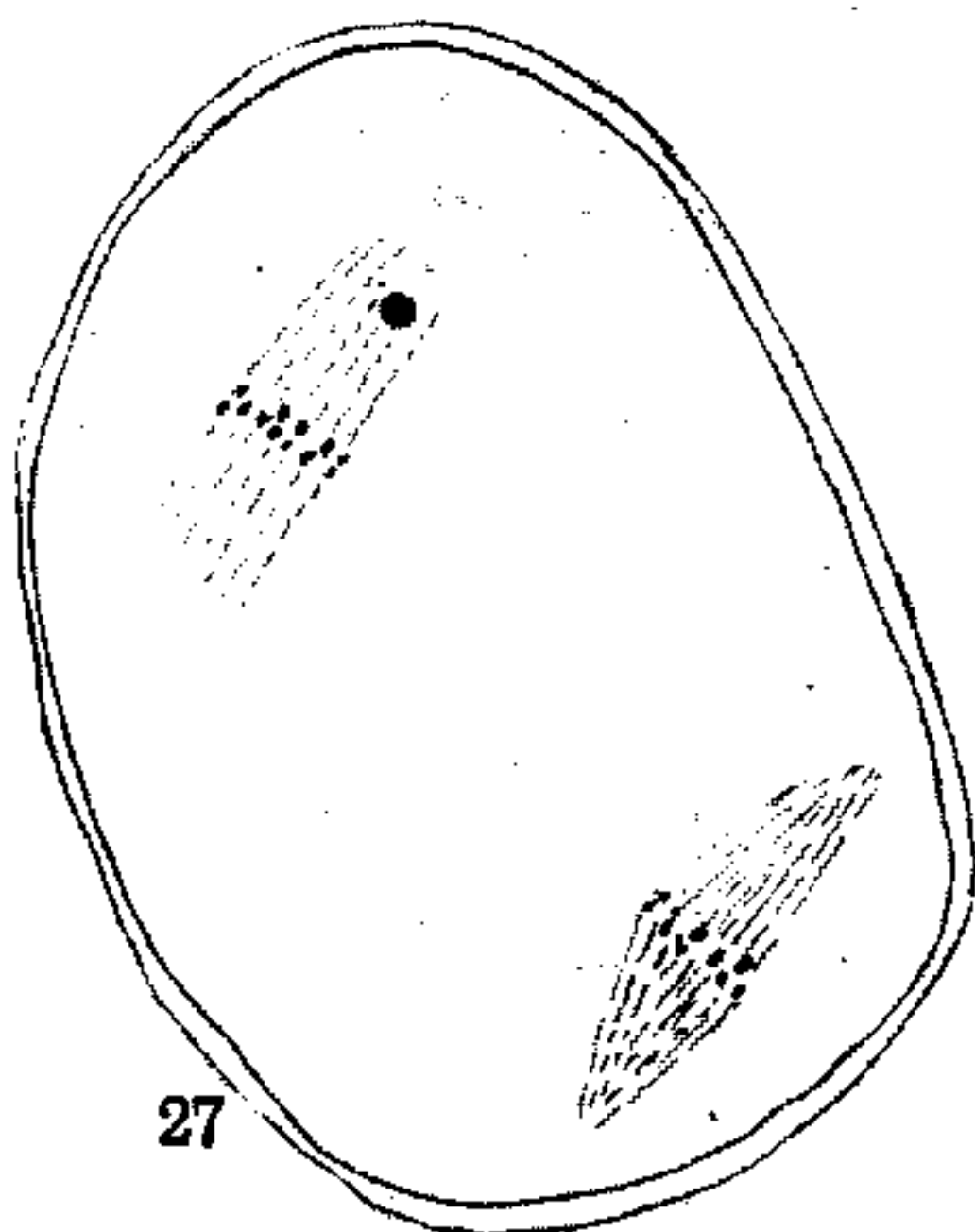
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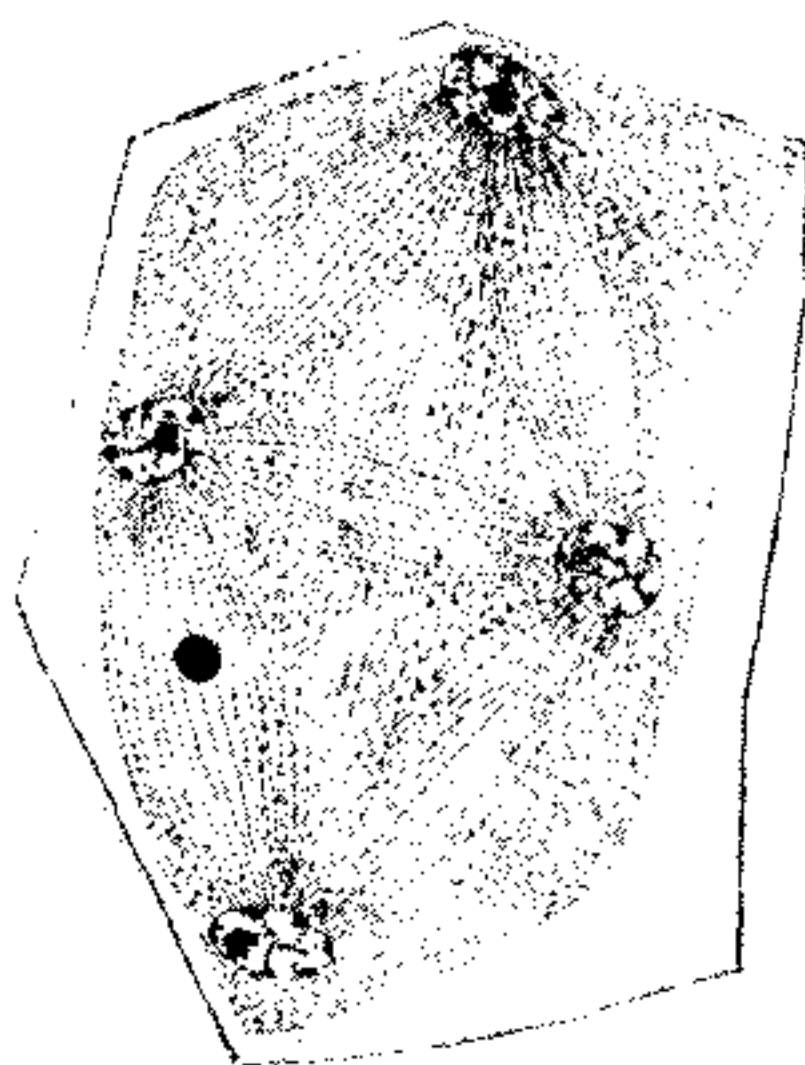
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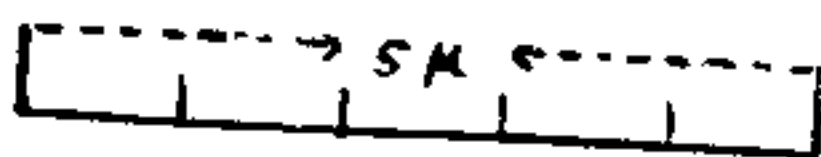
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nucleolus. In text figure 27, a nucleolus is seen in one of the metaphase plates. It may further be noted that, the nucleolus has already moved in the direction of the pole, ahead of the chromosomes. In text fig. 26, which represents anaphase II, two nucleolar bodies are seen, one in each spindle. It may also be observed from the same figure, that while the chromosomes have already commenced their anaphasic separation, the two persistent nucleoli are still seen in the neighbourhood of the equator.

Besides these, there are found a large number of other faintly staining bodies. These are not of the nature of starch grains as these are not stained with iodine solution. These are again likely to be only nucleolar particles. These are scattered within the cytoplasm and also in the region of the spindle (text fig. 25). Similar bodies have been observed by Latter (1926) in *Lathyrus odoratus*. According to Van Camp (1924), nucleolar fragments are sometimes met with in the spindle region. From such recorded instances, it would appear that the small particles which are occasionally met with, besides the persistent nucleolus, are merely the product of fragmentation of the nucleolus, before it disappears.

(d) *The Spindle* :—Even as there are found to be 2 kinds of PMCs., one exhibiting normal nucleolus and the other persistent nucleolus

it would appear that there are two kinds of PMCs. in respect of spindle formation also. For, while in one type, normal spindle behaviour was noticed, in the other, a behaviour totally different from the normal was found. This is described below :—

A. Ordinary bipolar spindles as are represented in text figures 4, 18 and 19.

B. Multipolar spindles as in figures 23, 24 and 25.

A. BIPOLAR SPINDLE :—The bipolar spindle which is met with in a large number of the pollen mother cells, fall into three distinct classes. (1) Bipolar spindle of the elongated type (fig. 10). (2) Bipolar spindle of the curved type (fig. 11). (3) Bipolar spindle of the short type, (figs. 5 and 6).

The spindle fibres are well formed and are easily distinguishable. They are achromatic and present varied appearance, with reference to their length, shape etc., In some cases, these fibres attain considerable length as a result of which, the telophasic nuclei come to lie just within the PMC wall on either side. Text figure 10, represents one of the PMCs. having such an elongated spindle. Standing in sharp contrast to this, are those in which the spindles are pretty short. These are seen in text figures 5 & 6. [Text figures 5, 6, 10 and 11 have all been drawn from the same slide under the same magnifica-

tion.] The relative lengths of the spindle fibres may be gathered from these figures. That there are found such contracted spindles, is further evidenced by examining text figure 9, which has also been drawn under the same magnification. In this are shown two interphase nuclei. (Care was taken to ensure that this is not a case of the polar view of the same). Considering the distance that separates the two nuclei, it is evident that these have been organized from those PMCs., which have developed only a short spindle. Text figure 11 represents a PMC with a long but curved spindle. Such crescent-shaped 'attractosomes' are merely regarded as artifact caused by the violent action of the fixative by Yasui (1941).

B. MULTIPOLAR SPINDLES :—Multipolar spindles have been quite frequent in their occurrence and recorded by several authors in a variety of plant materials belonging to the Gymnosperms and the Angiosperms. Timberlake (1900), studied in detail the formation of cell plates in higher plants. For his experimental material, he chose such plants as Onion *Larix* etc., and he found that, at the time when the chromosomes have arranged themselves at the equator, that is during the metaphase, the spindle is monaxial having only one axis. He further noted, that the fibres did not end in points but were blunt. At this stage, he was

able to recognise three different types of spindles :—1. The *connecting fibres*; 2. the *mantle fibres*; 3. the *radiating fibres*. According to Timberlake, the connecting fibres are those which lie entirely within the spindle and stretch across the equator. Mantles are those, which lie at right angles to the equator, within the spindle and are attached to the chromosomes. The connecting fibres and the mantle fibres together constitute the spindle proper. The radiating fibres are those which have only one of their ends free. In these, one end is usually centered around the pole while the other end merges into the peripheral cytoplasm. In a few case, even this fixed end has been found to cut across the spindle fibres and merge into the cytoplasm. Spindles in such cases will have both their ends free.

Multipolar spindles have also been recorded in Rice, (Kuwada 1910, Selim 1930 and Parthasarathy 1938). Parthasarathy's figures in Rice show, tripolar spindles and also transitional stages showing how these ultimately become bipolar. Such multipolarity has been met with both in the 1st and 2nd divisions. Besides these, the same author has described what are known as the 'compound spindles.' Here, some of the secondarily associated chromosomes develop spindles with distinctly separate poles. Mensinkai (1939) has also recorded the

occurrence of such 'compound spindles' in the mitotic metaphase of *Gladiolus tristis*. Besides these different types of spindle formation, accessory spindles are formed between the bivalents which are shot off into the surrounding cytoplasm, as has been shown for rice by Parthasarathy (1938 a), triploid *Urginea indica* and *Tridax procumbens* (Raghavan and Venkatasubban, 1940 and 1941).

In the present case, prior to the organization of the telophasic nuclei, 4 more accessory spindles were seen. These are shown in text figures 23, 24 and 25. Whether these are formed during the metaphase or only later, one cannot be definite. However, such spindles were not seen during the first metaphase. These accessory spindles, have both their ends fixed at the poles and at the equator. These radiate from either of the poles and stretch at an angle of approximately 45° , meeting at either end of the equator. The arrangement of the accessory spindles, present a typical appearance of a tetrad having six of those spindle fibres, such as have been figured for *Dahlia* (Wodehouse, 1931). A number of plates were examined, in order to ensure that the stage referred to, is not the 2nd telophase; for, the same appearance would be presented if by any chance, two of the telophasic nuclei have been cut off, leaving only their spindle connection. The fibres bear a superficial

resemblance to the radiating fibres, described by Timberlake (1900). However, these are entirely different since, these have both their ends not in a free condition, as would be required of the radiating fibres. That the ends are fixed and not in a free state was made sure by examining the preparation after cutting off the light. Therefore it is clear, that there is the occurrence of multipolarity and the formation of multipolar spindles. But multipolar spindles usually arise during diakinesis, prior to the disappearance of the nuclear membrane and with the onset of prometaphase the spindles assume the usual bipolarity. This is so in *Grevillea robusta* (Kausik, 1938). Cases similar to what has been found in *Kigelia pinnata* with regard to the spindles, have also been met with in the artificially raised interspecific hybrids of *Papaver* by Yasui (1931). Three different types of achromatic fibres were noticed by him in the F_1 plants. In addition, an unusual case of cytokinesis in the PMC, side by side with the normal ones, was also found.

II. (e) *Fusion of the 1st and 2nd telophase nuclei* :—In some of the pollen mother cells, the telophase nuclei are closely approximated and remain without any reference to the spindle fibres (Text fig. 12 and photomicrograph Pl. I Figs 5 & 6). It appears as though, that at the end of the telophase, the nuclei get themse-

lves detached from the spindle fibres and move towards each other. These may be mere preliminaries prior to their fusion and formation of diploid gametes. In the second telophase also, a similar behaviour was observed. In Text figure 14, two of the nuclei are quite close to each other, suggesting that they are likely to fuse, while the remaining two, appear to approach each other, since they have no connection with the spindle fibres. A persistent nucleolus is also seen.

II. (*f*) *Organization of Pollen tetrads* :— Pollen tetrads are formed according to isobilateral or tetrahedral plan. The isobilateral arrangement appears to be the more frequent of the two. The tetrads are cut off by a process of furrowing and three types are recognized. (1) In this type, the furrow originates at the periphery and gradually extends inwards, until it reaches the opposite side. In this way, two dyads are cut off as a preliminary to the formation of tetrads (Text figs. 29 and 30). Each one of the dyads in turn produces a furrow at the periphery, which gradually extends to the opposite side, thus resulting in the organization of the tetrads. The division of the dyads leading to the formation of tetrads, is non-simultaneous (Text fig. 31). (2) In this type, the furrow originates in the same manner as described above, but instead of producing two dyads first,

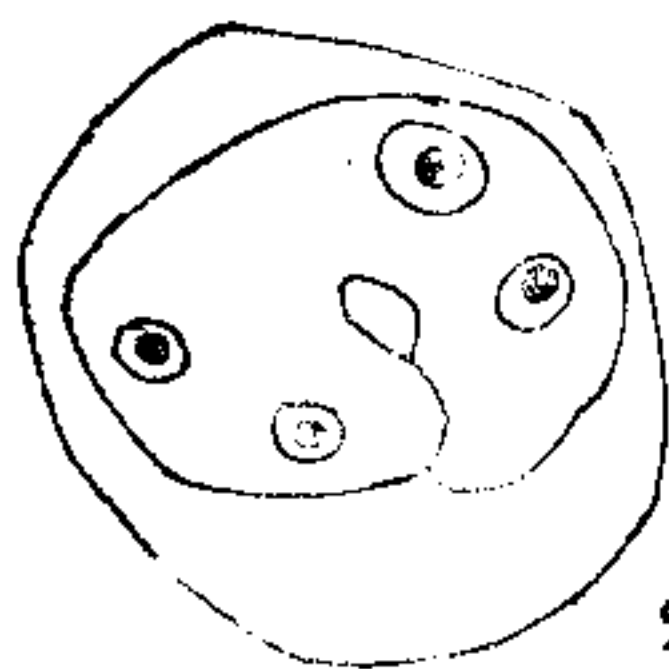
it cuts off individual tetrads one after another. In this way tetrads are organized non-simultaneously (Text figs. 32 and 33). Text figure 34 shows, the formation of tetrads according to the second type, besides revealing the presence of spindle fibres between two nuclei. The significance of the presence of spindle fibres at such a late stage as this, is discussed in Part II, under *Crescentia cujete*. (3) In the third type, tetrads are formed simultaneously. Here, the furrowing originates at the periphery, from more than one point, unlike the previous cases, where furrowing commenced from a single peripheral point and by their simultaneous ingrowth, met at the centre and produced tetrads, all at about the same time (Text fig. 35).

A large number of the tetrads formed, degenerate. Sometimes all the four tetrads may degenerate (Text fig. 36) or, three may degenerate while the other develop into a pollen grain (Text fig. 37) or, there may be formed two pollen grains out of the four (Text fig. 38).

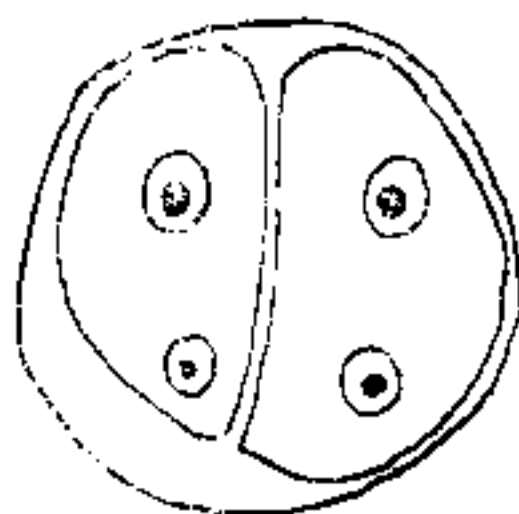
II. (g) *Cell plate formation* :—Cell plate formation appears to be more common in the Monocotyledonous plants than in the members of the Dicotyledonous group. However there are a few exceptions. For instance in the F_1 hybrid of *Papaver somnifera* $\times$ *P. orientale*, Yasui (1931) observed cell plate formation, in the first meiotic spindle. Yasui also states, that

Legend to text figures 29-38.

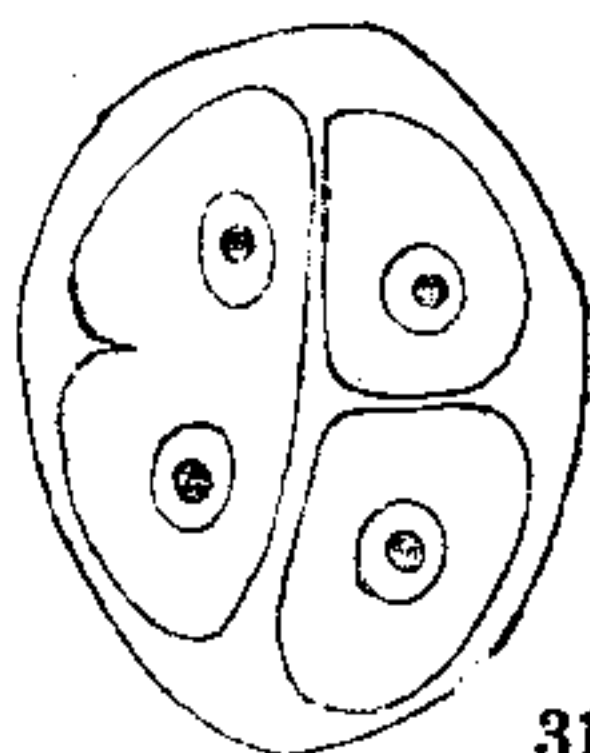
- Text fig. 29 : A dyad in the process of being formed by peripheral furrowing.
- „ 30 : Two dyads formed by the process of furrowing.
- „ 31 : Nonsimultaneous tetrad formation out of the dyads.
- „ 32 & 33 : Formation of tetrads by peripheral furrowing without the formation of dyads.
- „ 34 : Tetrads showing the presence of spindle fibres between 2 nuclei.
- „ 35 : Simultaneous tetrad formation.
- „ 36, 37 & 38 : Various stages of degeneration of Pollen tetrads.



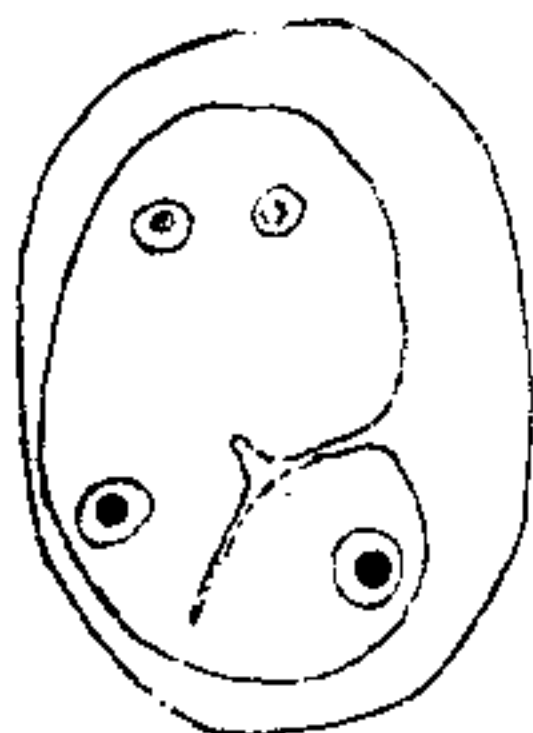
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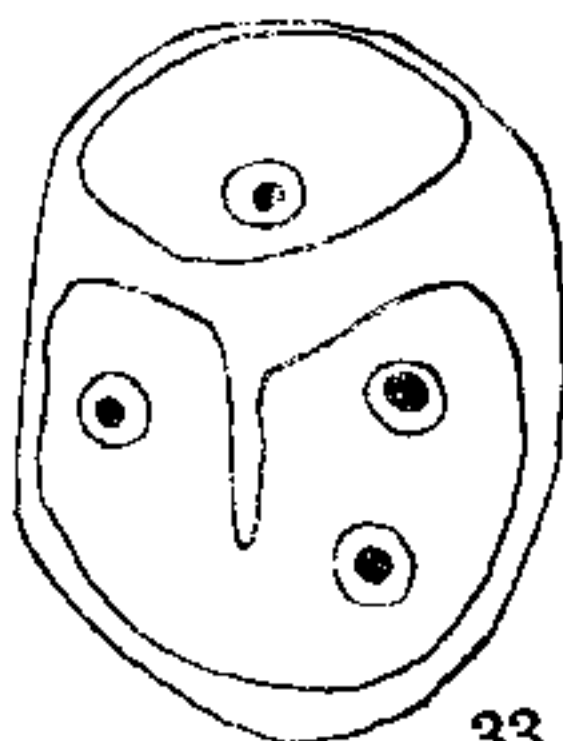
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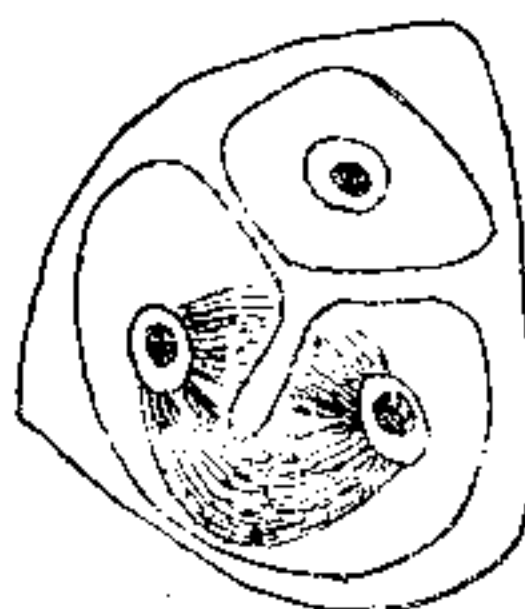
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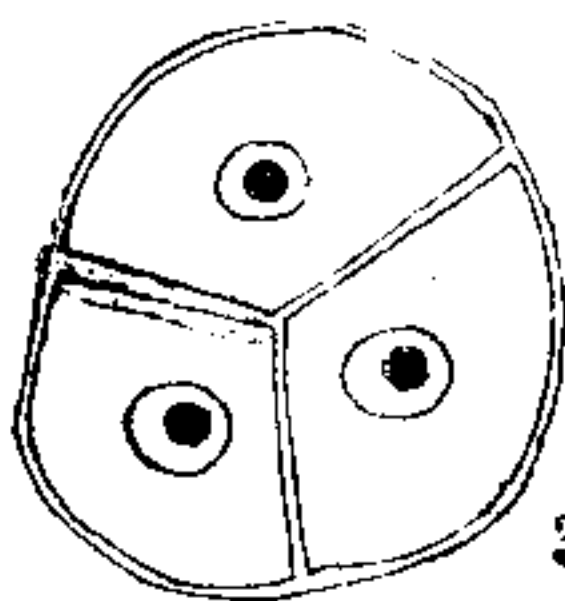
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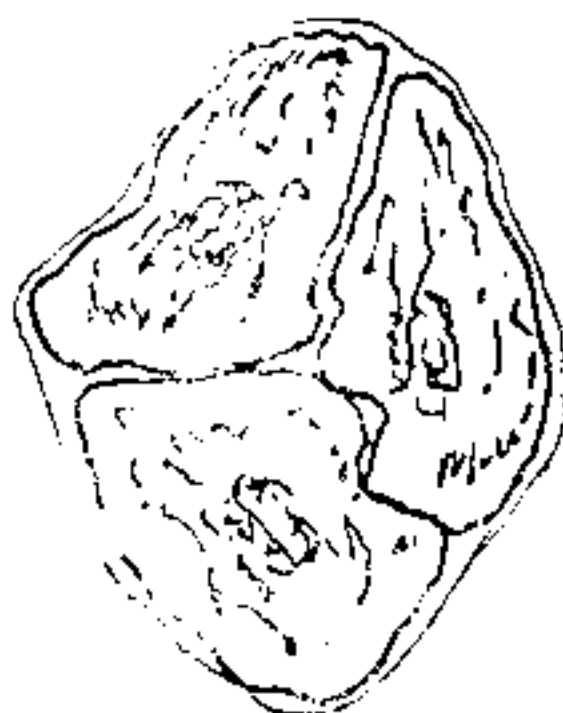
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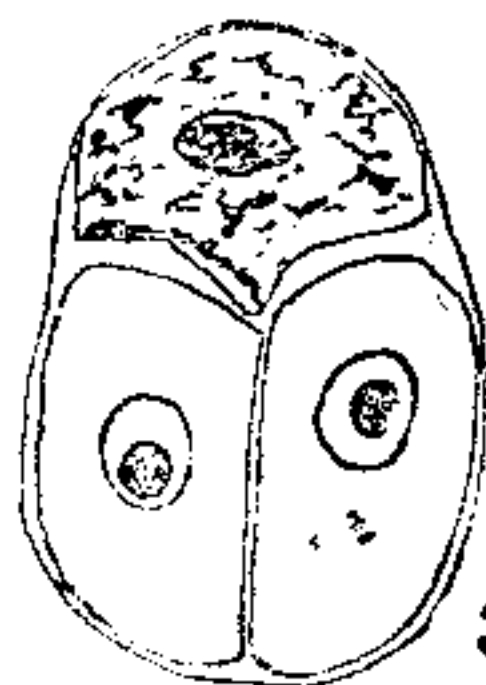
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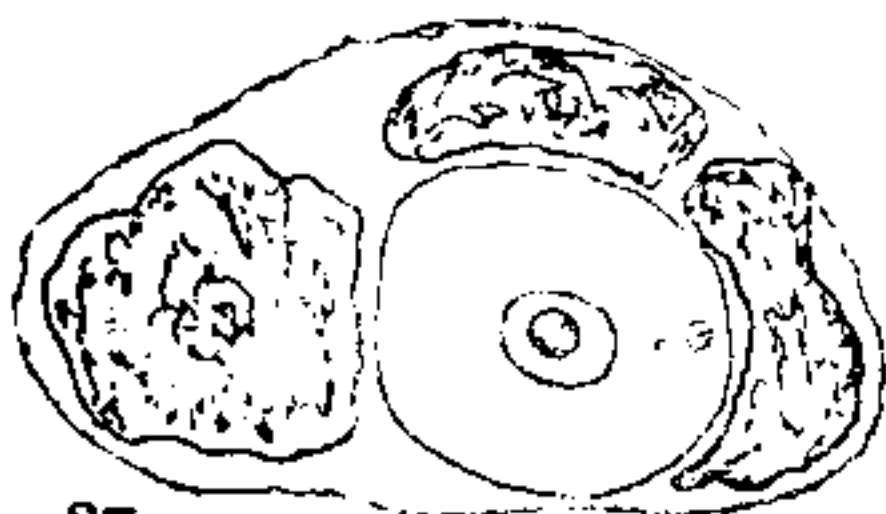
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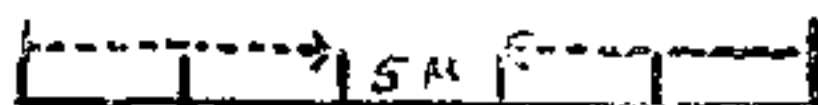
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38



37





similar cell plates are formed in the second meiotic division in *Crepis virens*. In the present case, cell plate formation was found in the second meiotic division similar to *Crepis virens* (Text fig. 14). It is interesting that, though the prevailing method of pollen formation is by furrowing, cell plate should be found also. The possibility of this structure being remnants of the spindle of the second division did not fail to receive consideration. But after a careful examination, these were found to be cell plates. This sets one thinking, whether there could not be any phylogenetic significance. It is commonly conceded that cell plate formation is more primitive than the furrowing process. It may well be, that what is seen in this case, is the vestigial presence of cell plate and this would indicate, that the furrowing process is to be derived from an ancestral cell plate process.

III. DISCUSSION

(a) *The nucleolus as a guide to ploidy* :—

It is now widely accepted that nucleoli are organized at telophase by definite bodies having their loci on the SAT-chromosomes. According to Heitz (1931), nucleoli originate in the satellite stalk or at the region of the secondary constriction and that the number, position and size of the nucleoli at the telophase, will depend upon the number, position and differential acti-

vity of these, on the loci of the SAT-chromosomes. From this, it follows that the number of nucleoli at the telophase, may be used as a guide for determining the number of SAT-chromosomes, in the chromosome complement of a given plant. Such an indirect method to find out the number of SAT-chromosomes in the complement, sometimes has to be resorted to, as in the majority of cases, the SAT-chromosomes are not visible, though theoretically speaking, every plant should have two or more of this type of chromosome.

The absence of the satellited chromosomes in many of the preparations, may partly be due to their extremely small size, or to other causes. Sometimes, the satellites fuse with the body of the chromosomes, thus resulting in their apparent absence. According to Navashin (1934), in a species cross of *Crepis*, though the parents had the SAT-chromosomes, the F_1 hybrid did not show the presence of these. Navashin calls this phenomenon 'amphiplasty' and the disappearance of the satellites in the F_1 generation is interpreted, as being due to the inability of the nucleolar body to function in a new environment or, due to competition in nucleolar organization among the nucleolar chromosomes (Navashin, 1927). A similar case of amphiplasty has been recorded in the hybrids between *Salix alba* and *Salix fragilis* by Wil-

kinson (1941). Loss of satellites may also be due to mutation and such mutations have been inferred to have taken place in rice varieties by Ramanujam (1937). In all these cases, the presence and the number of satellited chromosomes could be inferred by determining the number of nucleoli organized at telophase. If there are two nucleoli, at meiotic telophase, it would mean, that there are two SAT-chromosomes, 4 nucleoli would mean, 4 satellited chromosomes in the haploid genome.

Often, it is found that the chromosomes which are responsible for the organization of the nucleolus, remain attached to it during the prophase or even during diakinesis. Jacob (1940) in species of *Cassia*, Sikka (1940 a) in species of *Narcissus*, have found the satellited chromosomes remaining attached to the nucleolus, even during the prophase of somatic mitosis. Srinath (1939) similarly found in *Calceolaria* species, that the number of chromosomes attached to the nucleolus at prophase and the number of satellited chromosomes in the somatic cells was the same. In *Polanisia trachysperma* also, a similar attachment of the satellite chromosomes to the nucleolus was seen at prophase by Raghavan (1938 b). From such considerations, it would appear, that the number of chromosomes attached to the nucleolus at prophase or even at a later stage, would correspond to the number of SAT-chromosomes or

chromosomes with secondary constriction and also to the number of nucleoli formed at telophase. Thus there is a vast accumulation of data which would show a direct relationship between the satellited chromosomes, the number of nucleoli at telophase and the number of chromosomes attached to the nucleolus either during prophase or diakinesis.

The attachment of two bivalents to the nucleolus during diakinesis in the PMC., of *Kigelia pinnata*, would therefore indicate, that two nucleoli are to be found at telophase and that two satellited chromosomes or the same number of chromosomes with secondary constriction, are to be found in the monoploid complement. In many cases, two nucleoli were observed during telophase and the occurrence of two bivalents attached to the nucleolus during diakinesis confirms the observation regarding the number of nucleoli at telophase I. From this it would follow, that there should be 4 satellited or secondarily constricted chromosomes in the somatic complement. Since no secondarily constricted chromosomes were observed in the somatic metaphase plate, it is to be inferred that there are likely to be only 4 SAT. chromosomes.

de Mol (1928), as a result of his studies on the triploid and tetraploid *Hyacinthus*, arrived at the conclusion, that the number of nucleoli

at telophase is an index and guide to the ploidy of the species in question. Thus diploids were found to possess 2 nucleoli, triploids 3 and so on. de Mol's findings have been amply supported by many of the recent investigations on the nucleolus. So also Heitz's concept (1931) namely, that diploids have only 2 satellited chromosomes and 2 nucleoli at somatic telophase and the number of satellited chromosomes in a given chromosome complement, may be used as a guide to polyploidy. Thus Nandi (1936) found, the occurrence of 2 and 4 bivalents attached to the nucleolus in the secondary tetraploid and octoploid species of rice. Ramanujam (1937) found, that two and three bivalents were attached respectively to the nucleolus in the diploid and autotriploid rice. Resende (1936), examined as many as 102 Aloinae species and in 99 of these, he found in the somatic complements, 4 SAT-chromosomes and 4 nucleoli in the early telophase. The other 3 species showed 2 SAT-chromosomes and 2 nucleoli. This conformity of the number of SAT-chromosomes to nucleoli at telophase has been found true for 43 more species of Aloinae investigated by Satô (1938). Bhatia (1938), has also drawn attention to the fact, that in the somatic complements of wheat 4 SAT-chromosomes and 4 nucleoli were met with in a tertaploid species, while 6 SAT-chromosomes and 6 nucleoli were found in a hexaploid member of the same species.

It may be pointed out here, that de Mol's method of evaluation of polyploidy has its limitations too. In a number of cases, it has been shown, that neither satellites nor chromosomes with secondary constrictions, are concerned in the organization of the nucleolus. For instance chromosomes which are shot off into the cytoplasm during meiosis, sometimes organize themselves into micro-nucleus, with its own nucleolus. In such cases, the organization of the nucleolus cannot be attributed to the presence of either SAT-chromosome or a chromosome with secondary constriction. In maize, McClintock (1934) has shown, that the number of nucleoli at telophase does not correspond to the number of SAT-chromosomes. The nucleolar body in this case appears to undergo fragmentation as a result of which, there is a considerable increase in the number of nucleoli. According to Matsuura (1935, 1938) all chromosomes in a given species are capable of developing a nucleolus. However, some of these become specialized due to differential 'valency' and this brings about a competition among the chromosomes for the organization of the nucleolus. Sikka (1940 a), has reported that in a few species of *Narcissus* especially *N. poeticus*, *N. gracilis* and *N. tazetta*, the chromosomes with secondary constrictions, do not participate in the organization of the nucleolus. Jacob

(1940), from a study of the organization of the nucleolus in a number of species of *Cassia*, *Sesbania* etc., has drawn the inference that the nucleolar material is derived from the matrix of all the chromosomes. These are seen at telophase in the form of small globules or diffused particles dispersed in the nuclear sap or in the form of angular bodies. He regards the development of the nucleolus as a surface phenomenon. The attachment of satellited or secondarily constricted chromosomes to the nucleolus, is explained, on the basis, that it is at the loci of these, that a collection of the nucleolar particles takes place and hence the formation of the nucleolus around these.

Barring such exceptions, Heitz's and de Mol's concepts have been found to hold good for a number of cases and applying the same to *Kigelia pinnata*, the following inferences may be drawn.

From a study of the attachment of chromosomes to the nucleolus during diakinesis, the suggestion has already been made that 4 SAT-chromosomes ought to be present in the somatic complement of *Kigelia pinnata*. Arguing on the lines of Heitz and de Mol, the presence of 4 SAT-chromosomes would indicate that the species under consideration is a tetraploid. It may be assumed that this species had for its ancestor a 10-chromosomed species. If such an

ancestor is crossed with another, having the same chromosome number 10, but with a difference in the chromosomes due to structural changes or gene mutation, then, a hybrid with 20 chromosomes, will be the result. In this hybrid, there will be found a very low frequency of pairing, just as in the case of the cross between *Nicotiana Bigelovii* and *N. suaveolens* as recorded by Goodspeed and Clausen (1927), and consequently, it will be highly sterile. If however, in the hybrid, duplication of the chromosomes takes place, either by somatic doubling as in *Primula kewensis* (Newton and Pellew, 1929), or by the fusion of unreduced gametes as found by Karpechenko (1927, 1929), in the *Raphanobrassica* crosses, fertility will be restored and the species arising from this cross, becomes established. Such an origin of the 40-chromosomed members from a 10-chromosomed ancestor, is also supported from studies on secondary association, which topic is treated separately.

The study of the few pollen mother cells during diakinesis has shown, that there is no indication of any multivalent formation. This is also confirmed by a close examination of the first metaphase plates in side view. The absence of multivalents would strengthen the inference already drawn namely, that the species in question is an allotetraploid, rather than an autotetraploid. This is not surprising, since in spe-

ciation allo rather than autopolyploidy plays a more important rôle. Therefore, the inference is drawn that *Kigelia pinnata* is an amphidiploid derived from a 10-chromosomed ancestor, though it functions as a diploid.

III. (b) *Nucleolus : its persistence* :—

In normal cases, the nucleolus makes its appearance at telophase and disappears with the onset of the metaphase along with the nuclear membrane. According to Darlington (1937), "During diakinesis the nucleoli become detached from their organizers on the chromosome ends and as a rule gradually disappear. In some plants, they come to lie like a cap on the surface of the nucleus and at the end of the prophase, are extruded into the cytoplasm, where they degenerate."

However, cases are on record to show, that both the nuclear membrane and the nucleolus may persist upto metaphase and even to a later stage. In *Urginea, indica* (Raghavan and Venkatasubban, 1940), the nuclear membrane is seen to persist even during the metaphase, but not so the nucleolus. Persistent nucleolus has been frequently recorded in somatic mitosis. Frew and Bowen (1929) have cited a number of such cases, both in the lower and higher plants. These authors, have observed the nucleolus to persist in the root tip

mitosis of *Cucurbita pepo*. They found that, the nucleolus at first came to lie on the metaphase plate and assumed a dumb-bell shaped structure and then got pinched in the middle, thus getting divided into two—just like the separation of the buds in *Saccharomyces*. Each daughter half migrated to the opposite poles. They also noted that, the nucleolar bodies reached the poles ahead of the chromosomes. However, these were not included in the reconstruction of the daughter nuclei, but simply underwent fragmentation and diffused into the surrounding cytoplasm. From such a behaviour of the nucleolus, the authors came to the conclusion, that the spindle area is a region where, there are localized forces and these forces act upon the bodies which happen to lie in that region, regardless of their nature.

Zirkle (1928), from observations on *Zea mays*, adduced evidence that plastin forms part of the nucleolar substance and this passes into the chromosomes at mitosis, while the rest of the nucleolar substance divides at metaphase and the product of division passing to opposite poles. Thus an explanation was given as to the cause of the persistence of the nucleolus. As recorded by Frew and Bowen, (1929), Zirkle also observed, that the divided bits of the nucleolus reached the poles much earlier than the chromosomes, underwent fragmentation there and the

bits diffused into the peripheral cytoplasm, prior to disintegration. From such a behaviour of the nucleolus, Zirkle suggested a mechanism by which, hereditary stimuli could be passed on from the chromosomes to the organism. He also observed that plastin being electro-positive, change the electro-negative spireme into an electro positive chromatin complex. Thus the chromatin which had collected at the equatorial plate and which is away from the poles of the spindle, reverses its motion, consequent on the change of electric charge and thereby gets attracted towards the pole. Van Camp (1924) has also observed in detail, nucleolar fragmentation in the somatic mitosis of *Clivia miniata*. He has figured and described the passage of the nucleolar fragments through the spindle fibres to poles and their subsequent extrusion into the surrounding cytoplasm. Besides these instances, Yamaha and Sinoto (1925) have recorded a number of plants, belonging to both Monocotyledons and Dicotyledons, where the nucleolipersist. Recently, Ramanujam (1938) has observed a similar behaviour of the nucleolus, in a few somatic cells of *Oryza sativa*, (variety, T-24, Coimbatore). He found that the nucleolus lying between the chromosomes at the centre of the spindle, developed a constriction in the middle, thus assuming the shape of a dumb-bell prior to division into two, and each half moving to opposite poles. Depending upon

the orientation of the nucleolus, which was by no means uniform, it divided either into two equal parts or into two unequal parts. When the nucleolus was left outside the plate, it simply reached the pole, without undergoing any division. He also found that in all these cases, the nucleolus reached the pole earlier than the chromosomes, thus conforming to the previous observations. Ramanujam also confirms the previous view namely, that the division of the nucleolus at the equator and its movements to the poles were due to the presence of forces at the spindle region. *Oryza coarctata* Roxb also shows a persistent nucleolus during somatic mitosis (Parthasarathy, 1938 b). Mensinkai (1939), has reported the same phenomenon in the mitosis of *Gladiolus tristis*, *G. primulinus* and *G. dracocephalus*. In these, the nucleolus persists not only at metaphase, but also in the anaphase and even in the interphase. A number of species of *Calceolaria* also appear to exhibit this phenomenon (Srinath, 1939).

All these instances are cases of persistence of nucleoli during mitosis. It appears that their persistence during the heterotypic division, is a matter of rather infrequent occurrence. It has been found in the swede and turnip groups of *Brassica* (Catcheside, 1934). In these plants, Catcheside has come across several cases of nucleolar persistence during meiosis and he

considered such a behaviour of the nucleolus, as comparatively unimportant in the economy of the cell. Castetter (1925) found in a few varieties of *Melilotus alba*, the occurrence of four and two bodies respectively, in the homotypic and heterotypic divisions. These he regarded more in the nature of centrosomes. Beard (1937) came across certain bodies in the cytoplasm in the PMC., of *Echinocereus papillosus* and allied plants. These were noticed during the early prophase and diakinesis, as two spherical or oval bodies, at opposite ends of the cell. At the first Metaphase stage, these two bodies were seen to arrange themselves, on either side of the spindle. After the first Metaphase, there were found 4 such bodies, instead of two. These were observed during interkinesis, second Metaphase and second Telophase, but they disappeared at the end of second Telophase.

Frankel (1937), has recorded abnormal cases of nucleolar behaviour, in a few species of *Fritillaria*. In *F. obliqua*, there was found a 'cap nucleolus' during the zygotene stage. The 'cap nucleolus' later on, got detached and persisted as a solitary body during diakinesis, first Metaphase, Interphase, second Telophase and pollen tetrad stages. It was found that, the persistent nucleolus was included, in only one of the pollen grains formed. It is noteworthy that in this species, the persistent 'cap

nucleolus' remained as a solitary body throughout the meiotic divisions and that no such persistence of the nucleolus could be observed in mitosis. The same author found in *F. meleagris* var. *contorta*, two persistent nucleoli during the first metaphase.

In addition to these, Frankel has described, what are known as 'Telophase Globules'. These bodies were seen during early telophase onwards, in the PMC. of *F. citrina*. The 'Telophase Globules' sometimes, got themselves attached to the distal ends of the chromosomes, by a thread. In this condition, the chromosomes which were attached to these globules resembled very much those having trabants. According to the author, the 'Telophase Globules' represent the excretion of accessory chromosomal material.

Parthasarathy (1939), has also recorded a similar instance of the nucleolus persisting in the first metaphase of *Ehrharta calycina*. *Cicer arietinum* also exhibited a similar phenomenon (Iyengar, N. K., 1939). More recently Sikka (1940, b) found in the F_1 hybrid of *Brassica trilocularis* $\times$ *B. rapa*, a large number of the pollen mother cells, showing persistent nucleolus in the first metaphase and first anaphase. Subsequently, it fragmented into smaller bodies and escaped into the cytoplasm. Kumar

and Abraham (1942) in a recent publication, described what they call 'Secondary nucleolus.' These bodies were observed to arise as buds from the primary nucleolus and persisted throughout the meiotic divisions in the sterile as well as fertile plants of *Sesamum orientale*. In the cells of the young ovaries of sterile plant of the same species, a case of persistent nucleolus has also been recorded by the same authors.

Other possibilities were considered before arriving at the conclusion, that the spherical body seen in some of the PMCs., of *Kigelia pinnata*, present a case of persistent nucleolus. At first it was thought, that it might be a case of one of the nuclei of a multinucleate PMC., remaining in the resting condition. Such multinucleate PMCs., have been observed in *Lactuca* (Gates and Rees, 1921), *Zea mays* (Randolph and McClintock, 1926), in the hybrids of *Raphanus* $\times$ *Brassica* (Karpechenko, 1927), *Prunus avium* (Darlington, 1930), Rice varieties (Nandi, 1936) and in the common weed *Tridax procumbens* (Raghavan and Venkatasubban, 1940). Such multinucleate PMCs. have also been found occasionally in *Crescentia cujete*. Multinucleate PMC., may arise through cytomixis or by suppression of wall formation at the pre-meiotic mitosis. An extensive search was therefore made to find as to whether such multinucleate PMCs., are found in this material

also. None of the pollen mother cells examined revealed such a condition. The absence of such multinucleate PMC., precluded the possibility of the body under consideration being in the nature of a nucleus.

The possibility of the spherical body or bodies being in the nature of a chromosome or chromosomes, was considered next. In the somatic complement of *Kigelia pinnata*, there are found only 40 rod-shaped chromosomes. These are quite small and there is not much disparity in their size, even though they are not identical. The absence of big chromosomes in the somatic complement excludes the possibility that the very big spherical body seen in the PMC., could be one of the bivalents. Further, there are already found 20 bivalents in the PMC., excluding the spherical body. Therefore, the spherical body, cannot be in the nature of a bivalent.

Apart from such negative evidences, evidences of a positive kind were derived from a number of considerations. For instance Bailey (1920), found a colourless halo around the nucleolus, in the cells of the cambium and its derivatives. Such a colourless halo is also characteristic of the persistent bodies herein described. The behaviour of the persistent body under consideration, is very much like what has been

found in the case of the persistent nucleolus in *Cucurbita pepo*, described by Frew and Bowen (1929) and in *Zea mays* by Zirkle (1928). These cases are not identical with what is found in *Kigelia pinnata*; for, the observations of Frew and Bowen and Zirkle, refer to the nucleolus during mitosis, while in the present case it refers to meiosis. Despite this a certain degree of parallelism is noticeable. Zirkle and others have noticed, that the persistent nucleolus prior to its being extruded into the cytoplasm, invariably moved towards the poles. The same course namely via the pole has been adopted by the persistent bodies, under consideration. Further, the amitotic division and also fragmentation in the case of the persistent nucleolus, are also features characteristic of the body under consideration. The stain taken up by the persistent body and the nucleolus during the resting stage or diakinesis, is more or less comparable, though much weight could not be attached to this. All the same, this may be taken in conjunction with other pieces of evidence. The size of the body was measured during metaphase and anaphase and compared to that of the nucleolus, at various stages of meiosis.

From the table given in page 4, it is seen, that the average diameter of the nucleolus at the resting stage is 21.9 mm., in the prophase

and diakinesis, 12.5 mm., in the second telophase 10 mm., and in the pollen tetrad 13.95 mm. The diameter of the persistent bodies measured at Metaphase I and anaphase I is 9 mm. These bodies are only slightly smaller as compared to the nucleolus at diakenesis. So from the point of view of size also, the persistent bodies can favourably be compared to the nucleolus. From all these considerations, it is evident that there could be no doubt as to the nucleolar nature of the persistent bodies, often seen in the PMCs., of *Kigelia pinnata*.

From the same table, it may also be observed that the nucleolus undergoes a diminution in size, from the resting stage onwards. This is quite comprehensible since, the nucleolus is supposed to give out its contents to the chromatin matrix. It is also consistent with the idea, that the nucleolus contributes the matrix substance, to the developing chromosomes, from prophase to metaphase (Marshak, 1932, McClintock, 1934). Perhaps the whole of the nucleolar material is thus imparted to the chromatin threads, which become more and more conspicuous. When all the nucleolar material is thus exhausted, the nucleolus disappears. But Nandi (1937) found in different varieties of rice, there was at first an increase in the size of the nucleolus and then a decrease from the prophase to diakinesis.

The different behaviour of the nucleoli, would suggest the possibility of the existence of more than one type of nucleolus. According to Wilson (1928), there are two types of nucleoli; the Plasmosomes or the true nucleoli and Karyosomes or chromatin nucleoli. It is only the chromatin nucleolus that behaves as the store-house of chromatin material and from which, the developing spireme draws out its requirement. Wilson considers that the nucleolus may contain other substances than chromatin; for instance, it may contain plastin, in which case, it may behave in an entirely different manner and it would not contribute anything to the developing spireme. Gates (1932) is of opinion that there is no clear evidence to warrant the assumption that there are two types of nucleoli in the cells of higher plants. Francini (1938) from an exhaustive study of the root tips of *Papriopedilum spicerianum*, came to the conclusion, that the nucleolus consists of two parts; (1) a protein portion which is not stained by haemotoxylin and which remains the same in volume during the prophase. (2) A portion that takes up the stain and which diminishes in volume during the prophase stage. The composition of this has been found to be in the nature of sulphuric ester of a polysaccharide. This part of the nucleolus is stained deeply by Ruthenium Red. Jacob (1940) on the other hand found by Feulgen test, that the

nucleolus did not contain any protein. Mensinkai (1939) regarded that the nucleolus contained lipoids which may act as a fuel substance. Kumar and Abraham (1942) from nucleolar studies in the root tips and PMCs., of both fertile and sterile plants of *Sesamum orientale*, drew the inference that the 'nucleolus did not contribute any material to the chromosomes.'

The next question is, whether the persistent nucleoli play any part in the economy of the cell or as Catcheside (1934) has remarked, it is only an aberrant case and hence of no importance to the cell. There are conflicting evidences as regards the role of the nucleolus especially in the early literature. Zirkle (1928) was able to trace the passage of the plastin into the spireme. In *Pinus Strobis*, the same author (Zirkle, 1931), found that the nucleolar material was distributed throughout the entire length of the chromosomes. Schaede (1928) could not get any evidence for such diffusion of the nucleolar material, though he believed, that the nuclear threads took up the plastin, in an altered form. According to Frew and Bowen (1929), the nucleolus does not contribute anything at all to the chromosomes. Zirkle (1931) states, that it is not necessary that all the plastin or nucleolar material, should pass on to the developing spireme ; but often a residuum is left, which manifests itself in the form of a

persistent nucleolus, met with in mitosis and very infrequently in the heterotypic divisions. Since the detailed investigation in *Lathyrus odoratus* by Latter (1926), it is quite evident, that the nucleolar material is passed on to the developing spireme. The works of Heitz (1931), McClintock (1934) etc., have provided unmistakable evidence as to the participation of the nucleolus in the development of the chromatin matrix.

Various explanations have been given as to why the nucleolus persists in certain cases. Mensinkai (1939) interpreted the persistent nucleolus as having survived the last division and left out of the daughter nuclei so as to lie in the cytoplasm. An alternative explanation which he has given is based on the assumption, that the nucleolus contained lipoids, from which the persistent nucleolus might have arisen, during cell metabolism. But the author himself states that the second explanation is less probable.

In the present case, the view put forward by Zirkle namely, that not all the nucleolar material is passed on to the developing spireme and that a residuum is left behind, which persists in the subsequent stages of cell division, may hold good. There is also the likelihood of the nucleolus containing materials much in excess, to be transferred to the spireme. There may be another factor namely, time,

which may also cause the nucleolus to persist. For instance, if the nucleolus had no time for effecting a complete transfer of its material to the spireme, owing to a rapid succession of the various stages, either the whole of the nucleolus or part of it, will be left as such. A very significant fact which may be mentioned in this connection is that, while there is promiscuity in the cell division in various stages in the loculus of an anther, diakinetic stage alone, has been found to be wanting. Presumably, this is due to the fact that diakinesis is of a very short duration and appears as though it is skipped over. Thus there is a possibility of a time factor being involved in the transference of the nucleolar material to the spireme and when this is cut down, the transference of the nucleolar matter, is also proportionately reduced and the nucleolus, still containing sufficient material, may simply pass on to the next stage—metaphase, where, it persists for a time. It seems therefore likely, that plants in which diakinesis is of a very short duration, the nucleolus may be expected to persist. Of course further data are necessary before this can be generalized.

The persistent nucleolus has been observed to exhibit varied behaviour so far as its movements are concerned. In an undivided state, it is seen to move to the neighbourhood of the pole, much earlier than the chromosomes. When it

divides into two, each half may migrate in the opposite direction towards the poles or, one migrates to one pole while the other remains in the region of the equator. Even in an undivided state, the persistent nucleolus has been observed to take up a position near the equator, where it simply remains till telophase, without showing signs of any movement. In one case, the nucleolar body took a lateral course and reached one end of the equator, along one of the accessory spindles.

The factors which are responsible for causing such a variety of movement, are not clear. Factors involved in the movement of even the chromosomes themselves, have not been sufficiently well understood and the nature of the forces involved, is still a matter of controversy and uncertainty. According to the earlier investigators in this field, chromosomes move to the poles due to various forces, such as the contraction of the spindle fibres, electro-magnetic attraction or repulsion, currents in the cytoplasm, pulsating or oscillatory bodies situated at the poles etc. In all these cases, the chromosomes are believed to be carried in a passive manner, to their destination, by one of these forces. However according to Belar (1928), bivalents can make movements of their own, though such a faculty is wanting in the case of the univalents.

due to repulsion between spindle attachments. During anaphase, the movement may be due to the stretching up of the spindle fibres. That chromosomes can have their own propulsive activity, is also to be seen from the evidence gained with the spermatocytes of *Sciaria* (Metz, 1936). Here, the segregation of the chromosomes took place not in a bipolar field as it is the case in normal division, but in a unipolar field. In the spermatocytes of *Sciaria*, there is formed only one spindle. Curiously enough, the segregation of the chromosomes took place in the usual way. Segregation of the chromosomes took place in this monocentric mitosis, by the actual movement of the chromosomes, in opposite directions. This activity, is certainly in co-ordination with other forces. The 'insertion body' situated at the base of the spindle fibre, is perhaps responsible for effecting this propulsion of the chromosomes. The matrix sheath substance, which is presumably of a non-genic nature, may also aid in bringing about chromosome movement, by altering the viscosity of the protoplasm, in front and behind the moving chromosomes. But such instances are few and far between. In many cases, chromosome movement takes place due to magnetic attraction or repulsion, set up by cytoplasmic currents, in the spindle region. In a few cases, chromosome movement may also be due to the

contraction of the spindle fibres, especially the 'mantles' of Timberlake (1900), as the chromosomes are directly attached to these.

As regards the movement of the persistent nucleolus, it may be due to, either one, or several of the forces, cited previously, which bring about the movement of the chromosomes. Mensinkai (1939) regarded, the division and migration of the persistent nucleolus to the poles, as due to the stretching of the spindle fibres. Since no connection is to be found between the nucleolus and the spindle fibre, it is unlikely, that the contraction or the despiralisation of the spindle, is concerned in the movement of the nucleolus. The other alternatives then are, either cytoplasmic currents or magnetic fields. The fact that sometimes, the persistent nucleolus remains at the equator, while in others, it moves towards the poles, would suggest that these movements may be due to cytoplasmic currents. Further, it would appear, that the spindle region is one of localized forces and when a body happens to be in that region of forces, it is carried away, irrespective of the nature of the body, provided it is free to move and not attached to anything like the spindle fibre. That the forces in the spindle region are not uniformly distributed but localized, is evident from the behaviour of these bodies, namely, a few remain at the equator while others move to the poles. The initial position

assumed by the nucleolus on the spindle region, also determines at least in part, the course of its further behaviour.

In this connection may be mentioned, the observations of Macky (1931), on the deformation and breaking of water drops, effected through the medium of strong electric fields. Some of his photographs showing the deformations, bear a very close resemblance to the persistent nucleolus, especially when it is about to divide. At first sight, it would appear that the forces that cause the division of the nucleolus and the water drop, are more or less identical, as similar pictures are obtained in both cases. That is, both in the water drop and in the nucleolus, the central part gets very much thinned out, assuming the shape of a dumb-bell. The water drops employed by Macky were sufficiently small (0.085–0.26 cm. radius). According to his observations, water drops of a given radius r cms., when exposed to increasing fields, become elongated until at a definite field given in volts per cm. by $F\sqrt{r} = 3875$, become unstable and filaments are drawn out from the ends. The above formula was applied to the case of the persistent nucleolus. Its radius was determined and the pole distance was calculated by taking the average of a dozen readings. The result arrived at however, show that the stretching of the nucleolus prior to

division, cannot be due to pure electric fields, as in the case of the water drop. The magnitude of the electric field is of a very high order and hence cannot be applicable in the case of cell.

Why the persistent nucleolus should move ahead of the chromosomes, is not clear. During metaphase, the chromosomes are attached to the spindle fibres and since contraction of the fibres takes place only a little later, the chromosomes are prevented from being carried away by the forces, at the spindle area, operating at that time. On the other hand, the nucleolus, is not attached, and is free to move. Therefore it is likely reach the pole earlier than the chromosomes. The same may be explained with the help of the Electro-magnetic theory of nuclear division (Kuwada and Sugimoto, 1928). According to these authors, the chromatin becomes changed into electro-positive from electro-negative. Such a change is possible since according to Zirkle (1928), the plastin material gets uniformly coated on the chromosome. Plastin is electro-positive and as such, the charge on the chromatin is also changed. Such a change takes place at about the time of the disappearance of the nuclear membrane. But in the case of the persistent nucleolus, the plastin remains as such, since it is not transferred to the chromosomes and hence remains as a highly electro-positive body. Thus, the electric charge

prevailing on the surface of the persistent nucleolus is opposite to the charge at the region of the poles. On account of this, the nucleolus is likely get attracted towards the pole.

On the other hand, the chromosomes could not have changed their electric charge. This may be due to entire absence of plastin on the surface of the chromosomes as the bulk of it remains only in the nucleolus, or, the plastin is found only in such small quantities, that it does not effectively alter the nature of the electric charge of the chromosomes. From this it will follow, that the electric charge on the surface of the chromosomes and that at the region of the poles is similar and hence the chromosomes are not attracted towards the poles. The differential behaviour of the chromosomes and the persistent nucleolus, may thus be explained with reference to the nature of the electric charge that prevails on these bodies and at the region of the poles. It may be pointed out here, that in normal cases, where there is no persistence of the nucleolus, the chromosomes change their electric charge due to the transference of the plastin from the nucleolus.

III. (c) *The Spindle* :—It has already been noted that supernumerary spindles are found in some of the PMCs. The function of these is

not clear. Generally, these spindles serve for the movement of a few chromosomes, mostly in the nature of univalents, fragments etc., and help in their being organized into micronuclei. In this case however, no such function could be ascribed. Multipolar spindles also provide some evidence regarding the compound nature of the spindles in general.

It is likely that spindle formation is governed by the presence of definite particles in a diffused state in the cytoplasm. In normal cases these particles may be assumed to show a tendency to arrange themselves in an axial position, with reference to the equator. The formation of minor spindles in the organization of micronuclei, out of the univalents or fragments, can also be explained on such an assumption. The formation of accessory spindles late in the metaphase, or the formation of bipolar spindles out of multipolar ones, can also be explained on the basis, that there are bodies—the pole determiners or pole organizing bodies in the cell. If it is assumed that these ‘pole organizers’ can diffuse from one place to another in the cytoplasm and that poles could be organized when a sufficient concentration of this organizer is reached, all the above observed phenomena could be explained.

There is also some significance with regard to the short spindles, which have already been

described. Contracted spindles have been induced by the application of various chemicals to the living cell, (Shigenaga 1937). Short spindles have been induced in the petal cell of *Tradescantia*, by the application of chloral hydrate solution of the following strength ; 0.25 % ; 0.3 % ; 0.4 % and 1 %. When the cells were kept immersed in such a solution, it was found that they passed from the metaphase stage to the anaphase stage, but the spindle was so much contracted, that the two daughter chromosome groups, were soon merged into one. Shigenaga concluded, that a decrease in the amount of water, played the main role in causing abnormal nuclear and cell divisions. Again he points out "this dehydration is regarded as the main cause of producing di-diploid giant nucleus or binucleate cell." These experiments clearly show the ease with which short spindles can be formed, namely, by the simple process of dehydration. The advantage that accrues as a result of such short spindles is that, the two telophasic nuclei are organized in close proximity. Such a juxtaposition of the interphase nuclei is not without any significance ; for, this may be the first step leading to the fusion of the interphase nuclei or it may be, that fusion would take place during the second metaphase. If the interphase nuclei are placed farther apart as will be the case with elongated spindles, then there is not much chance

for the fusion of the two anaphase nuclei or the two metaphase plates owing to spatial limitation, unless it is assumed, that the nuclei at these stages can actually migrate towards each other. But it is much easier for effecting such a fusion, if the interphase nuclei are placed at close proximity. The result of the fusion either at first interphase or during metaphase II would be the same, namely, the formation of diploid gametes. Such gametes in *Kigelia pinnata* would carry 40 chromosomes instead of the usual 20. Formation of diploid gametes as outlined above, is an important process since it is an important means, in the production of polyploid forms. No doubt, there are also other methods by which such an evolution could be achieved.

That fusion of interphase nuclei is likely to take place in this species can be inferred, not only from the occurrence of such short spindles, but also from another source of evidence. In a few cases prior to the organization of the telophase nuclei, connection was severed between the nuclei and the spindle fibres, with the result, that the nuclei concerned were free to move. The spindle fibres in such cases were seen to have no relation at all with the two nuclei. A similar thing was also noticed during the second telophase, [text fig. 14]. Similar and identical cases have been recently reported in

a parthenogenetically derived seedling of sugar cane POJ 2725, (Janaki Ammal. 1941).

Though there is sufficient evidence to infer that fusion takes place between the first interphase nuclei, there is no indication as to the fusion of the second metaphase plates as suggested previously. This may be due to the fact, that sufficient number of PMCs. were not examined and that the frequency of such fusion plates is comparatively small. Müntzing (1933) has observed fusion taking place between the 2nd metaphase plates in the triploid forms of *Solanum tuberosum*. Newton (1928) as a result of his studies on the F_1 hybrids of *Digitalis ambigua* $\times$ *Digitalis purpurea*, formulated a scheme by which diploid gametes are formed. In his first scheme, he stated that the first division may be normal and during the second division the chromosomes at metaphase were closely placed and these ultimately fused resulting in a diploid gamete. In his second scheme, fusion took place even during the 1st anaphase or interphase.

While studying the cytology of the F_1 hybrid of *Brassica Tournfortii* $\times$ *B. trilobularis*, Sikka (1940 b.) has recorded a number of interesting abnormalities, regarding the spindles. Besides the occurrence of supernumerary spindles due to the presence of univalents,

he has observed in a few cases, the fusion of the two homotypic spindles, especially when they happen to lie parallel and close to each other, thus resulting in the formation of two anaphasic groups, instead of the usual 4. This would naturally give rise to dyads from which diploid gametes can be expected. Similar fusion of the anaphasic spindles has also been recorded for *Raphano-Brassica* (Karpechenko, 1928) and for *Galeopsis* (Müntzing 1930).

The above instances clearly show the widespread occurrence of diploid gametes, in a variety of plants. In *Kigelia pinnata*, from the data on hand, it may be said that, diploid gamete formation is likely to take place either through the fusion of the interphase nuclei or through the fusion of the telophase nuclei, at the end of the second division. Though actual diploid gametes have not been isolated, the fusion of the nuclei at the 1st and second telophases, would lead to the inference, that such gametes ought to be found in this species.

III. (d) In conclusion it may be said that the attachement of two bivalents to the nucleolus at diakinesis, the occurrence of secondary association in this and allied species, the anaphasic irregularity leading probably to non-disjunction, the abnormal spindle formation, and the persistent nucleolus during the 1st and

2nd divisions, would point to the polyploid and hybrid nature of *Kigelia pinnata*. The nucleolar behaviour and the absence of multivalent formation would suggest that this species is an allo-polyploid, though functioning as a diploid.

V. SUMMARY

The somatic and meiotic chromosome numbers for *Kigelia pinnata* have been determined for the first time and found to be $2n = 40$ and $n = 20$.

Meiosis has been described in detail.

Two bivalents were found to be attached to the nucleolus at diakinesis. The nucleolus and its organization is discussed in the light of recent discoveries in the field as indicative of the ploidy of the species.

The nucleolus was found to persist throughout meiosis; the behaviour of this persistent nucleolus has been described in detail. The various causes leading to the persistence, have been discussed, in the light of observations made and views expressed by previous workers. It is suggested that it may be due to the excess of material which the nucleolus contained and the very short duration of diakinesis:

The migration of the persistent nucleolus ahead of the chromosomes at metaphase I, is explained on the basis of the Electro-magnetic theory.

Besides normal bipolar spindles, multipolar spindles, crescent-shaped spindles and short spindles have been observed. The features leading to these, and the possible significance of such abnormal spindles have been discussed, especially from the point of view of the origin of diploid gametes.

Fusion of the first and second telophase nuclei has been noted. This would also indicate the prevalence of diploid gametes.

From a detailed study of meiosis, it is suggested that *Kigelia pinnata* is an allo-tetraploid though functioning as a diploid and that it is derived from a 10-chromosomed ancestral form.



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Explanation of Plate I Figures 1-10

- Fig. 1. Diakinesis showing the attachment of two bivalents to the nucleolus. $\times 2700$.
- „ 2. Metaphase I. $\times 2700$.
- „ 3. Metaphase I showing one persistent nucleolus. $\times 1800$.
- „ 4. Anaphase I with a number of laggards forming a chain. Same as text fig. 7. $\times 1800$.
- „ 5. Telephase I, the two nuclei show a tendency to fuse. A stage earlier to what is seen in fig. 6. $\times 1800$.
- „ 6. Telophase I showing the fusion of the two nuclei. The spindle fibres are also seen by the side of the nuclei. $\times 1800$. Same as text fig. 12.
- „ 7. Anaphase I, with one persistent nucleolus lying near about the equator. $\times 2700$.
- „ 8. Anaphase I with one persistent nucleolus. Note that this has reached the pole earlier than the chromosomes. $\times 2700$.
- „ 9. Metaphase I with two unequal persistent nucleoli migrating to opposite poles. Note that the chromosomes are still at the equator. $\times 1800$. Same as text fig. 18.
- „ 10. First telophase, showing multipolar spindles and a single persistent nucleolus extruded into the cytoplasm. at A. $\times 1800$. Same as text fig. 25.

PLATE I. Figs. 1-5



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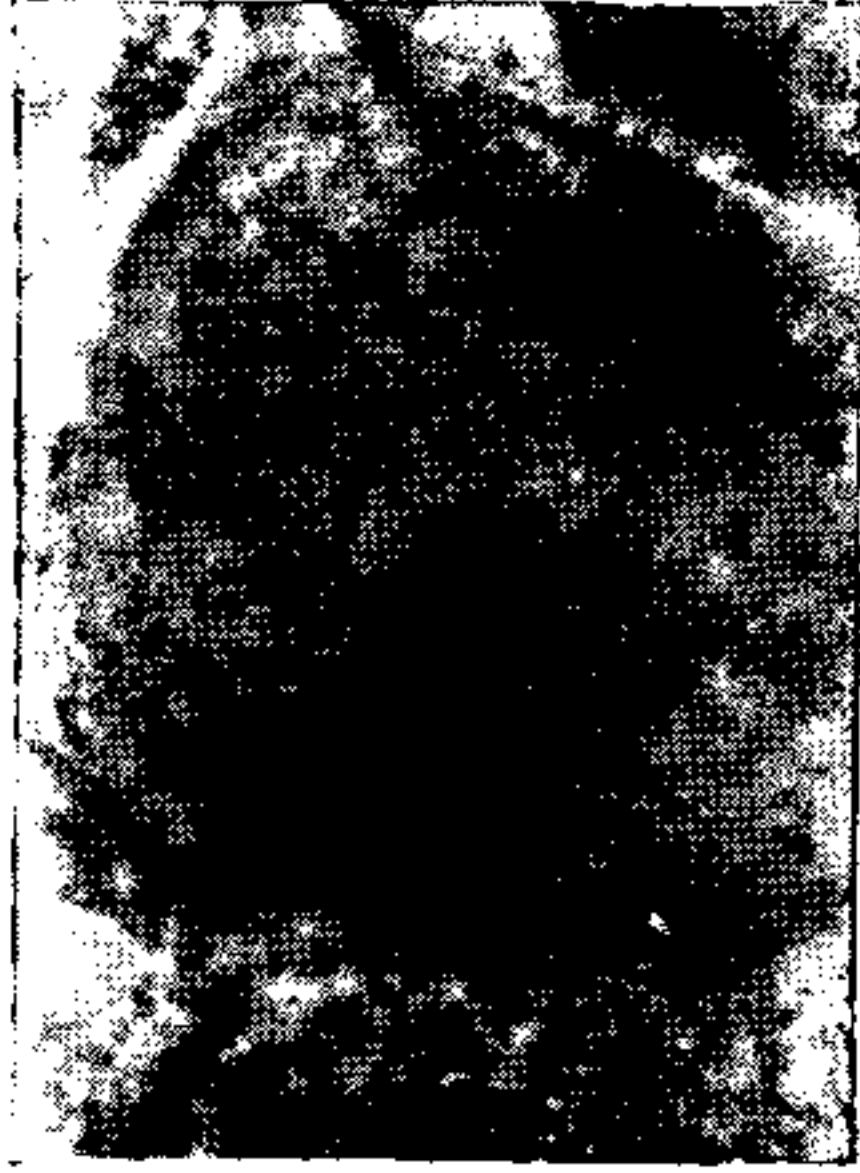
PLATE I. Fig. 6-10



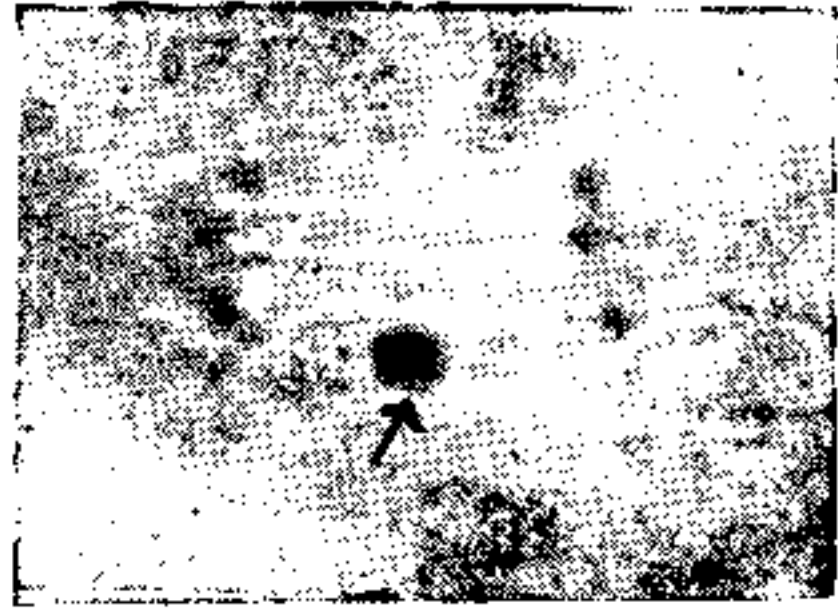
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PART II

THE CYTOLOGY OF CRESCENTIA CUJETE LINN. AND RELATED GENERA WITH SPECIAL REFERENCE TO THE PHENOMENON ✓ OF SECONDARY ASSOCIATION*

(With 1 plate and 105 figures in the text)

* Part II of a thesis approved for the Degree of Doctor of
Philosophy of the Annamalai University. ✓

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I. INTRODUCTION

CYTOLOGICAL studies in this family appear to be very meagre. Duggar (1899) was the first to give some cytological details regarding the development of the pollen grain and the embryo-sac in *Bignonia venusta*. de Vilmorin et Simonet (1927) studied *Tecoma Tagliabuana* and found the haploid number to be $n = 20$. Sugiura (1936) determined the chromosome number for *Incarvillea grandiflora* ($2n = 18$), *I. Delavayi* ($2n = 18$) and *Tecoma Smithii* ($n = 19$, $2n = 38$). Raghavan and Venkatasubban (1940 a) found, the meiotic chromosome number for *Spathodea campanulata* to be $n = 13$. Excepting for such stray chromosome counts, no further cytological information for the members of this family could be obtained. This may be partly due to the extreme unsuitability of the material for cytological studies. Duggar (1899) has also pointed out this. The large number of very small sized chromosomes and the erratic manner in which countable plates are obtained, even though fixation is done after a preliminary examination under aceto-carmin, are some of the obvious difficulties met with. Despite these obstacles, meiosis in detail was followed in a few members like *Crescentia Cujete*, *Tabebuia pentaphylla*, *Millingtonia hortensis*, *Parmentiera cerifera*, *Tecoma capensis*.

and *Tecoma stans*. Since there is not much variation in the reduction division in these, a detailed description is given only for *Crescentia Cujete* and for the rest, only the minimum details have been included. In almost all these, the phenomenon of secondary association was met with ; but a detailed study of this was made only on a few like *Crescentia Cujete*, *Tabebuia pentaphylla* and *Millingtonia hortensis*.

II. MATERIALS AND METHODS

Excepting *Parmentiera cerifera* which had to be obtained from the Botanical Garden, Sibpur the rest of the materials were found growing in the campus of the Annamalai University. The cytological technique does not call for any special mention. After a preliminary examination in aceto-carmin to determine the right stage of development of the PMC., the anthers in thin slices were fixed in Navashin's fluid for 24 hours. Materials prefixed in Carnoy's fluid gave decidedly better results. After washing and dehydration, the material was finally embedded in paraffin wax of 56°C M.P. Chloroform was employed as the solvent for paraffin. Sections were cut at a thickness of 14-16 microns. Slides were stained in both Iron alum Hæmatoxylin and Newton's iodine genetian violet. The latter gave better results especially for secondary association studies.

III. MEIOSIS: IN CRESCENTIA CUJETE

A large number of Pollen mother cells was found in each anther locus and this is due to the repeated division of the sporogenous tissue both anticlinally and periclinally. The same feature has been observed in *Kigelia pinnata*, *Parmentiera cerifera* and *Stereospermum chelonoides*. On the other hand there were only a few PMCs. in *Phyllarthron comorense*, *Tecoma capensis* and *Millingtonia hortensis*, and these were arranged in one or two layers. The PMC., in *Crescentia Cujete* was fairly big and had a polygonal outline, when it was in a state of division. It also remained adherent to other PMCs. till about the formation of pollen tetrads. Usually, the PMCs., lose their angularity and separate from one another during reduction divisions, though in the present case it has been found otherwise. Further, the different PMCs., in one and the same locus were found in different stages of reduction division.

At DIAKINESIS, 20 bivalents were seen (text fig. 1). Out of the 20 bivalents, 2 were of the ring type and the rest of the rod type with only one terminal chiasma. Since there were not enough pollen mother cells at this stage of meiosis, the frequency of the rod to ring bivalents could not be estimated. Multivalents were not seen in the cells examined.

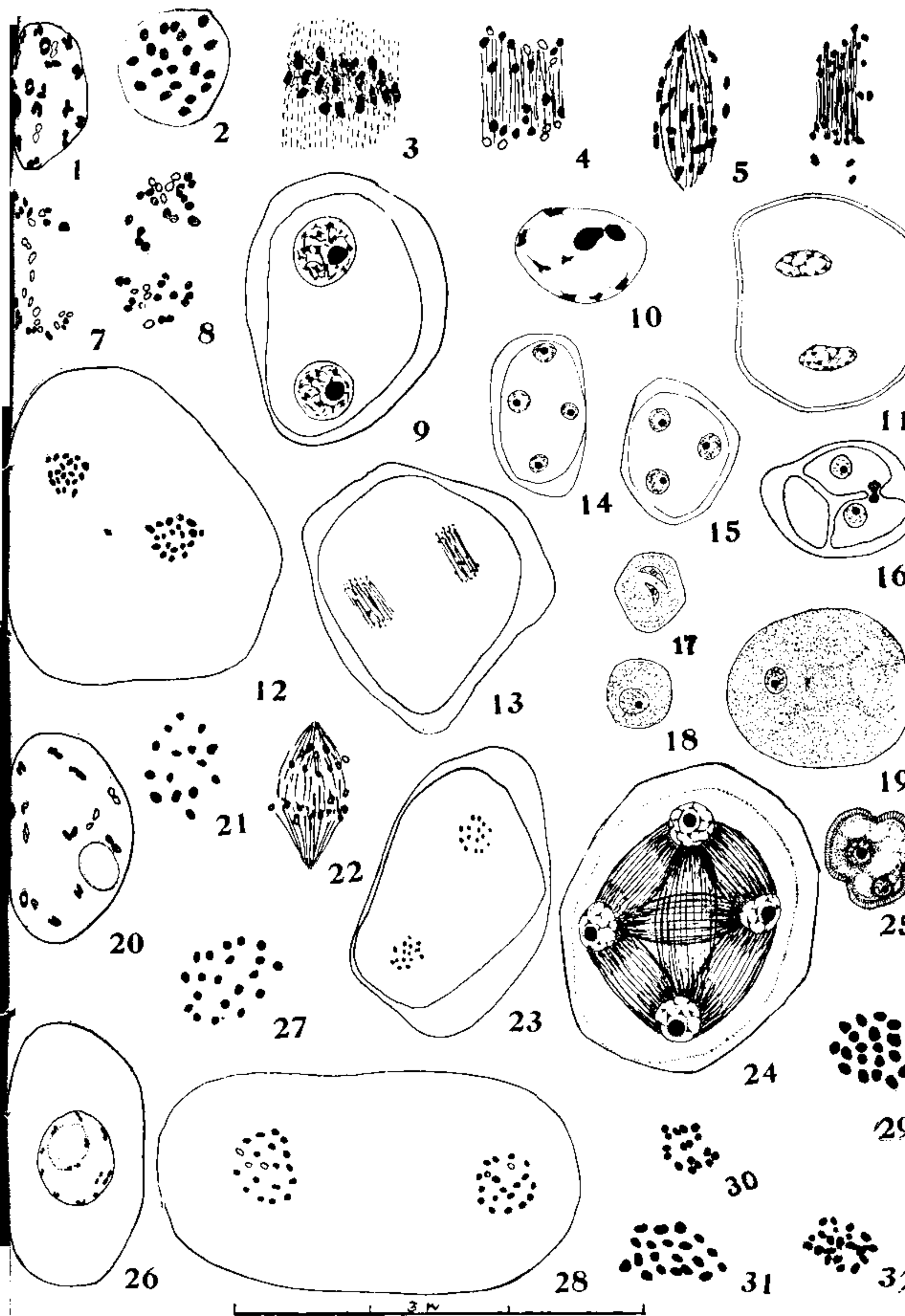
At METAPHASE I, 20 gemini were seen (Text fig. 2). This is the expected number, since in the somatic mitosis 40 chromosomes have been observed. About 100 different PMCs., at this stage were examined and in all these the number 20 was found to be constant. The 20 bivalents were seen in groups of two's and three's (text figs. 36-54). The association met with in this case is not a primary one. This can be seen by examining the first metaphase plate in side view (text fig. 3), where multivalents if any could be recognized easily. In this case multivalents were observed neither during diakinesis nor during metaphase I. Therefore the groupings observed, were in the nature of secondary association. Detailed account regarding this phenomenon is given elsewhere.

The FIRST ANAPHASE has been found to be quite normal but in a few cases, lagging chromosomes were observed (text figs 4, 5, 6 & 7). In fig. 7, three lagging chromosomes were seen, of which one has orientated itself approximately at right angles to the direction of the spindle fibres. The same condition has also been met with in anaphase II (text fig. 13). Where lagging takes place, it is the smallest chromosome that is left behind. Or, in those cases where multivalents are formed, the univalents generally lag. Occasionally fragments may also behave in this manner, as has been found

Explanation of text figures (1-32)

1. Diakinesis in the PMC of *Crescentia Cujete* $\times 3600$.
2. Metaphase I in *Crescentia*, $\times 3600$.
3. „ side view $\times 3600$.
4. Anaphase I, *Crescentia*, $\times 3600$.
- 5 & 6. Anaphase I, showing random distribution, $\times 3600$.
7. Anaphase I, showing 4 lagging chromosomes, one of which shows an orientation at right angles to the spindle, $\times 3600$.
8. Anaphase I, showing persistence of secondary association, $\times 3600$.
9. First interphase showing the spherical nuclei, $\times 1800$.
10. First telophase nucleus showing, 2 nucleoli and fibre, $\times 3600$.
11. First early telophase showing the flattened nuclei, $\times 1800$.
12. Metaphase II in *Crescentia*, exhibiting 20/20 distribution, $\times 2700$.
13. Anaphase II, showing laggards and spindles lying parallel, $\times 1800$.
- 14 & 15. Tetrads showing isobilateral and tetrahedral arrangement, $\times 900$.
16. Pollen tetrad exhibiting cytomixis; note the absence of a nucleus in one of the tetrads, $\times 900$ (same as Photomicrograph. Pl. 1. fig. 3)
17. Binucleate PMC; two crescent-shaped nuclei are seen $\times 750$ (same as photomicrograph, Pl. 1, fig. 4).

18. Normal uninucleate PMC for comparison of size $\times 750$.
19. A giant PMC of *Crescentia* $\times 750$ (same as microphot. Pl. 1, fig. 5).
20. PMC of *Millingtonia hortensis* in diakinesis $\times 3600$.
21. First metaphase in *Millingtonia* showing 15 bivalents, $\times 3600$.
22. First anaphase in *Millingtonia* showing 15 bivalents, $\times 3600$.
23. Second metaphase showing 15/15 arrangement, $\times 1800$.
24. Second telophase, showing 6 spindles, $\times 1800$.
25. Pollen grain of *Millingtonia* at the time of shedding, $\times 900$.
26. PMC of *Tabebuia pentaphylla* in diakinesis, $\times 1800$.
27. PMC of *Tabebuia pentaphylla* MI $\times 3600$.
28. " " " MII $\times 3600$.
29. PMC of *Bignonia magnifica* in the first metaphase, $\times 3600$.
30. PMC of *Tecoma capensis* in the first metaphase, $\times 3600$.
31. PMC of *Stereospermum chelonoides*, one of the MII plates, $\times 3600$.
32. PMC of *Tecoma stans* showing the first metaphase plate, $\times 3600$.





in X-rayed *Capsicum* (Raghavan and Venkatasubban, 1940 c). In the present case, the lagging chromosomes are to be regarded as the smallest chromosomes within the complement. Since lagging of chromosomes is only of sporadic occurrence in this case, no great consideration need be attached as to the causes underlying this. Besides these laggards, there was found another irregularity in anaphase I. During the anaphasic separation, the chromosomes were seen scattered all over the spindle (text fig. 5). The scattering in this case was somewhat symmetrical. However, in another case the scattering of the chromosomes has been more to one side, producing an asymmetric form (text fig. 6). Similar scattering of the anaphasic chromosomes has been found in *Kigelia pinnata* and in the second anaphase of *Stereospermum chelonoides* (text fig. 33). In one of the first anaphase plates examined, the chromosomes were found in groups of 2 suggesting the persistence of secondary association, seen in metaphase I (text fig. 8). However, Catcheside (1937) has stated that "With the commencement of anaphase I, repulsion between secondarily paired bivalents sets in intensely, so that by mid anaphase I nearly all the secondary pairing has disappeared. It is completely gone by telophase I". Raghavan (1938) in *Gynandropsis pentaphylla* found that in anaphase I, secondary association is maintained to the same extent as

in first metaphase. Iyengar (1939) found in *Cicer arietinum*, that in certain cases secondary association persisted in anaphase while in the majority of cases it disappeared. In the present case also, the majority of the PMCs., did not show association during anaphase I, except a few. The persistence of secondary association during anaphase I, may interfere with the segregation of chromosomes especially when groups of three are involved.

As soon as anaphasic separation is over and the chromosomes reach both the poles, telophasic nuclei are reconstructed out of these chromosomes. In the early telophase the two nuclei formed are quite flat as can be seen in text fig. 11 and photomicrograph, Pl. I. fig. 1. Catcheside (1937) has observed, the same flattened appearance of the early telophasic nuclei in *Brassica oleracea*. While describing them he says, "it is at first nearly flat or strictly, concavo-convex." In this case the concavo-convexity is not as prominent as the flattened appearance. The chromosomes exhibited the usual x-shaped configuration and appeared to be connected by very thin strands or fibres. These fibres became somewhat prominent as telophase advanced. The telophasic nuclei also exhibited the presence of two nucleoli as can be seen in text fig. 10. During interphase or interkinesis, the two telophasic nuclei appear to undergo an

increase in volume. At any rate the flat nuclei assumed a spherical shape (Text fig. 9 and photomicrograph, Pl. 1 fig. 2). The chromosomes were peripherally arranged as at diakinesis and some of the interphase nuclei also exhibited the presence of two nucleoli, seen during early or mid telophase (photomicrograph Pl. 1 fig. 2). The interphase seems to be of an appreciable duration as several of the PMCs were observed in this stage of meiosis.

During METAPHASE II, the chromosomes were seen in groups of two's and three's as in metaphase I, (text fig. 52-54). In other words, secondary association is seen to persist even during metaphase II. A few investigators are of opinion, that secondary association is seen much better in metaphase II, than in metaphase I. For instance Nandi (1936) in *Oryza sativa* found such a state of affairs. Müntzing (1933) has also observed a similar condition in *Solanum tuberosum*. However, Richharia (1937) found in *Raphanus sativus*, that secondary pairing was less prominent in M II than in M I. He suggested that "the attraction which weld the chromosomes together in M I, gradually diminishes as the process advances so that some associations are now likely to be very loose and no longer evident." In the present case, while there is unmistakable evidence of secondary association in M II, it cannot be said that it is better exhi-

bited at this stage than in M I. Perhaps the size of the chromosomes during metaphase II, is responsible for this deceptive appearance.

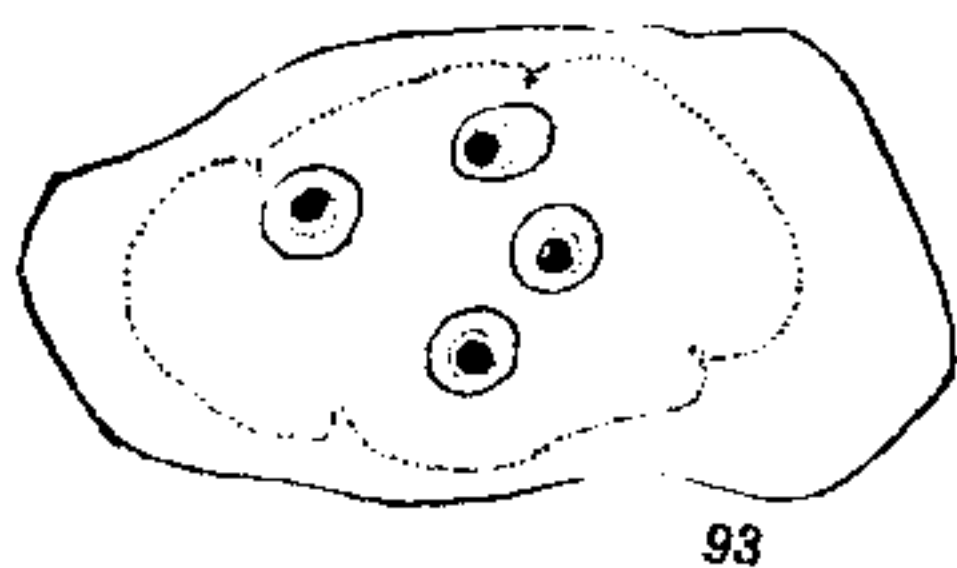
In the second metaphase the 20/20 distribution of the chromosomes was seen (text fig. 12). Second anaphase was found to be normal except that in a few instances, laggards were observed (text fig. 13). The two homotypic spindles may lie parallel as in text fig. 13 or they may get turned at right angles to each other or sometimes even obliquely. Depending upon the position of the two spindles, tetrads were organized either tetrahedrally or isobilaterally, (text figs 14 and 15). The tetrahedral arrangement was the one that prevailed in the majority of cases.

Tetrad organization :—Extensive degeneration of pollen grains has been observed in this species. Degeneration may set in at all stages of pollen development. However, it appeared to be at its peak at the time of pollen tetrad organization. The degenerated tetrads took a jet black stain with Iron-alum Haematoxylin and proper differentiation in these, was almost impossible.

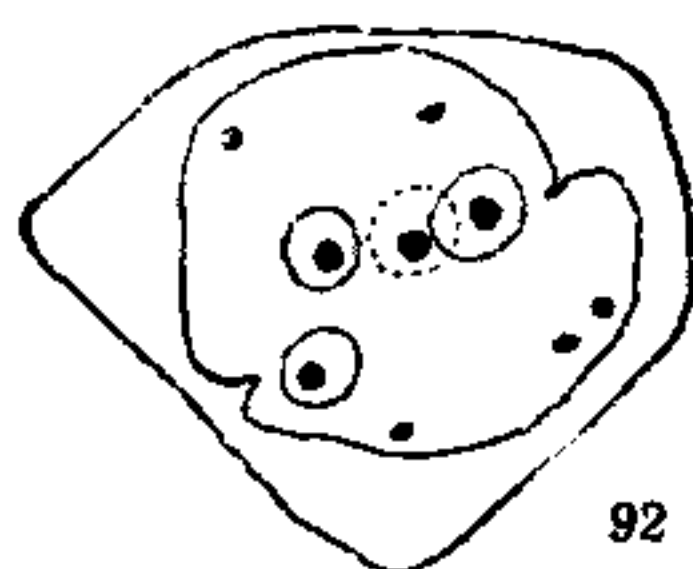
The unaffected pollen mother cells, after the usual divisions, were organized into tetrads. Tetrads were formed by the process of furrowing. Furrows started at 3 or 4 points at the periphery of the cell, depending upon whether the

Legend to text figures 92-97

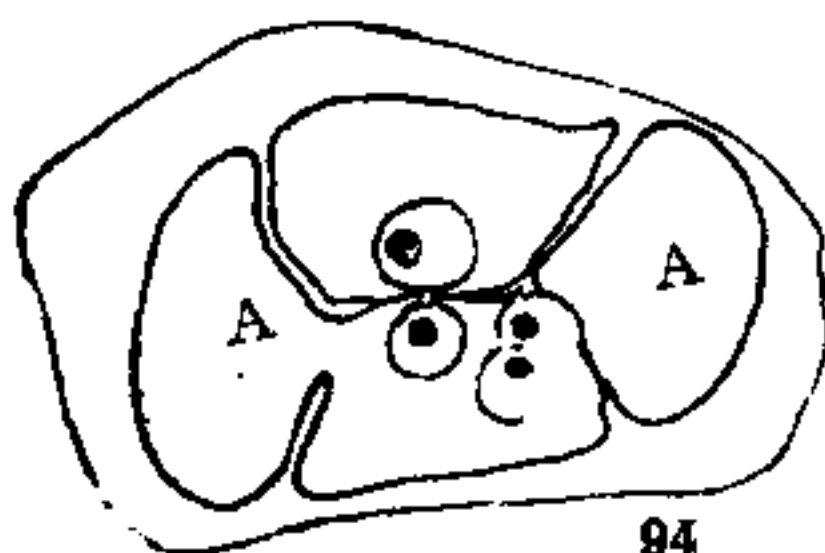
- Text fig. 92 : Cruciate type of tetrad formation (Tetrad formation in *Crescentia Cujete*) showing the beginning of peripheral furrows at four points and the 4 nuclei tending to move towards the centre.
- „ 93 : Tripartite type of pollen tetrad in *Crescentia Cujete* showing migration of nuclei towards the centre.
- „ 94 : Cruciate type of pollen tetrads exhibiting the diagonally developed furrow ; note the presence of 4 nuclei at the centre even though cytokinesis is almost to be completed. The cells A, A' are likely to become enucleate.
- „ 95 : Tripartite type of pollen tetrad in the same material showing 3 furrows and the nuclei at the centre.
- „ 96 : Degeneration of the microsporo-cyte with supernumerary nuclei.
- „ 97 : Tetrads formed according to the tetrahedral plan ; note that in one of the tetrads nuclear matter is degenerating leaving a single nucleus intact.



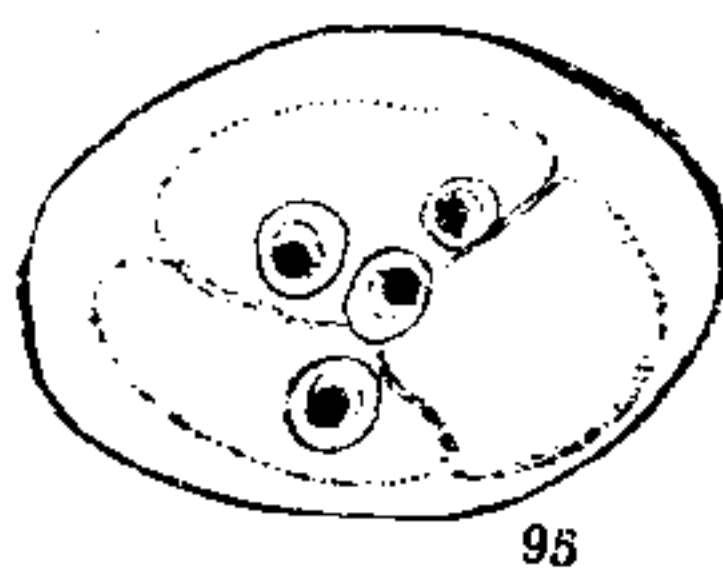
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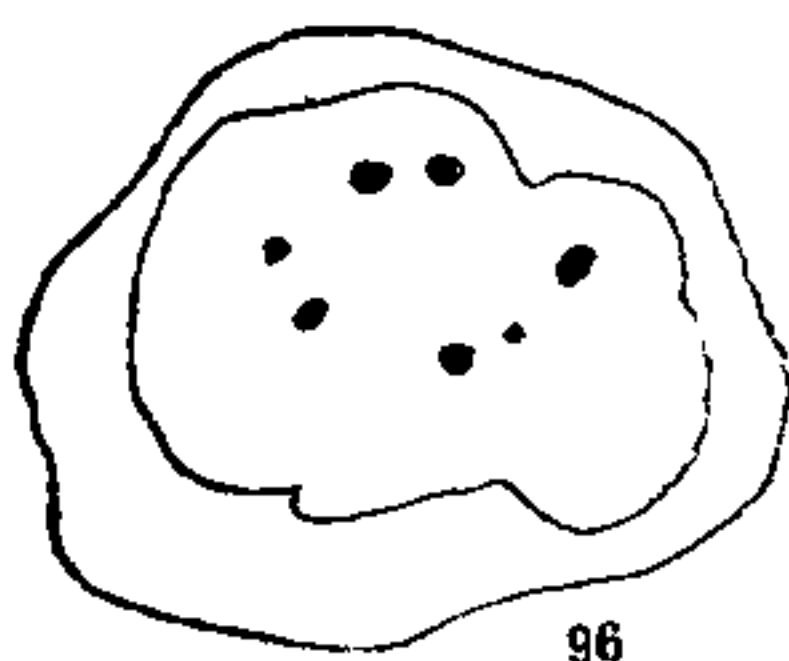
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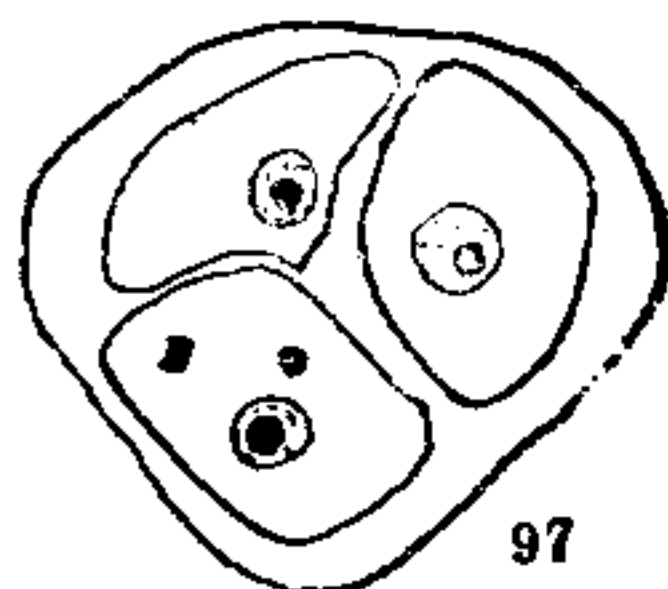
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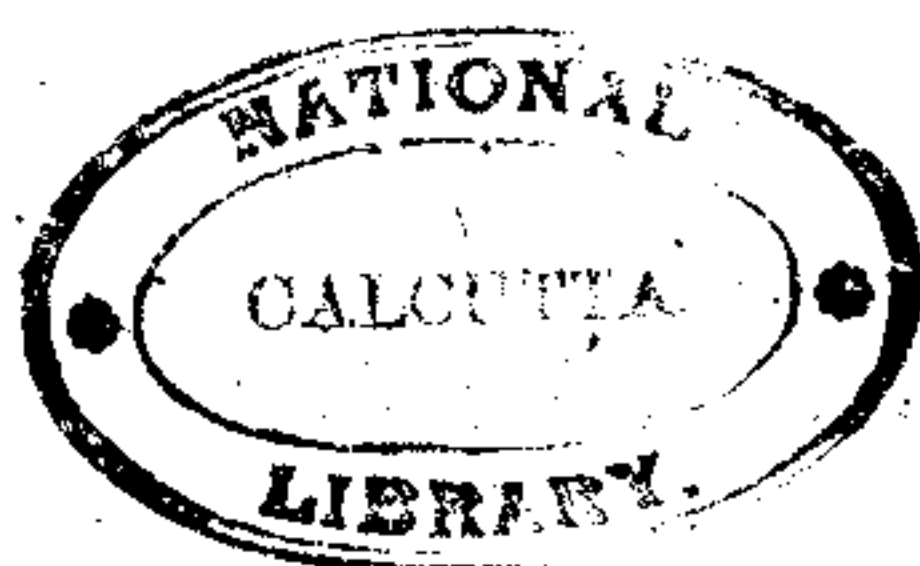


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microsporocytes are going to be developed according to the tripartite or cruciate type. At about the same time, the 4 nuclei migrated towards the centre of the cell (text figs 92 and 93). At first sight, it would appear as though the 4 nuclei are going to fuse in pairs; but no such fusion has been observed in any of the preparations. Now, what were merely depressions at the periphery, gradually and simultaneously made inroads into the centre of the cell. Thus in the cruciate type, four diagonally placed furrows were seen (text fig. 94). In the tetrahedral type, only 3 furrows were visible (text fig 95). It can also be observed from text figs 94 and 95, that the 4 nuclei are still at the centre, even though cytokinesis is almost over. Unless the nuclei migrate once again to their respective places at the corners of the cell, it will be likely that some of the microsporocytes will be devoid of a nucleus while others may have one or more than one. In text fig. 94, it is possible that two of the microsporocytes will be enucleate, one uninucleate while the remaining one, 3 nucleate. The enucleate tetrad will naturally degenerate. Tetrads containing more than one nucleus will also sometimes get degenerated (text fig. 96), or, only the extra nucleus or nuclei would be eliminated by a process of disintegration of the chromatin matter (text fig. 97.)

It is interesting to note that in this species, spindle fibres though they were conspicuous

during the first and second anaphases, were not to be seen at the commencement of cytokinesis. On the other hand in *Kigelia pinnata* (Venkatasubban, 1943) it has been observed, that the spindle fibres persisted even at a stage when furrowing was about to be over. It would appear that the spindle fibres by their persistence during cytokinesis as in *Kigelia pinnata*, keep the nuclei away from the path of the furrow and thereby ensure, that each one of the tetrads formed will have one nucleus. The absence of enucleate tetrads in *Kigelia pinnata* and their presence in such large numbers in *Crescentia Cujete*, will give some evidence to this view. The absence of spindle fibres connecting the nuclei during cytokinesis in *Crescentia Cujete*, may be one of the causes for the degeneration of a large number of tetrads, observed.

More than the absence of the spindle fibres during cytokinesis, it is the migration of the nuclei towards the centre of the cell, that is directly responsible for the formation of enucleate and multinucleate pollen tetrads. The cause of this migration cannot easily be explained. It may be that, the spindle fibres in their stretched condition kept apart the two attached nuclei; but when the spindle fibres gradually contracted and disappeared, the two nuclei might have sprung to the centre of the cell. It is also likely that, the nuclei can move about

within the cell of their own accord. Such movements or migrations of the nuclei have been observed in *Impatiens pallida* (Smith, 1935).

IV. MEIOTIC ABNORMALITIES IN *Crescentia Cujete* :—

(a) *Cytomixis* : Though many would consider cytomixis, an abnormality, its wide occurrence should make one think, that it is quite a normal feature. It appears to be quite characteristic of hybrids. The first report of this phenomenon was made by Gates (1911) in *Oenothera*. Since then, this has been met with and described for a large number of cases. Katterman (1933) has given a detailed treatment of this subject and has cited all relevant literature regarding the same. Matsuura (1935a) has also made a special study of this phenomenon. Cytomixis when present, usually manifests itself during the prophase stages. Its occurrence at later stages, is rather infrequent. However in *Tridax procumbens* (Raghavan and Venkatasubban, 1941) it has been found to occur during the whole of the meiotic cycle. In a *Nicotiana* hybrid, from a cross between *N. glutinosa* and *N. tabacum*, Raghavan and Srinivasan A. R., (1941), found a slightly modified form of cytomixis. Here, instead of the bivalents or univalents being extruded into the adjacent cell as is generally the case, a wad of chromatin material was involved.

In the present case, only very few PMCs., exhibited this phenomenon. This was found to occur after the organization of the tetrads (text fig. 16 and photomicrograph Pl 1. fig. 3). Cytomixis at such a late stage as this, is rather unusual. Here also as in *Nicotiana* hybrids, the extruded chromatin mass formed a wad. Probably an entire nucleus was involved in this process; for, while the nuclei were seen in two out of the 3 tetrads (text fig. 16), the third tetrad was without a nucleus. Such enucleate tetrads were quite frequent in their occurrence in *Crescentia Cujete*. This is probably due to the fact that, at the time of furrowing, the nucleus was not in position so as to be included in the cytoplasm. Very likely, it is this extra nucleus that has been extruded between the two normal tetrads. It may be mentioned in this connection, that enucleate pollen mother cells have been observed by Matsuura (1935b) in *Mitrastemon Yamamotoi* Mak. Such enucleate pollen mother cell or pollen tetrad, will merely degenerate.

(b) MULTINUCLEATE POLLEN MOTHER CELL:— Bi-nucleate PMCs., were seen in a few cases (Text fig. 17 and photomicrograph Pl 1. fig. 4). By the side of this is shown a normal PMC., (text fig. 18) for comparison. Apparently, there is no difference in size between the two. This would mean that the binucleate condition may be due to failure of

wall formation during the pre-meiotic mitosis. If it is a case of fusion of two of the PMCs, then the size of the resulting syncyte should be about double that of the normal uni-nucleate pollen mother cell. In fact Matsuura (1935a) observed that in *Phacellanthus tubiflorus*, actual fusion taking place between adjacent pollen mother cells during synezeisis and after the disappearance of the wall separating these, a giant PMC., was formed, which was found to be twice the size of the normal pollen mother cell. He also found, that such giant cells usually degenerated without undergoing any reduction division, unless one of the nuclei was eliminated as a result of degeneration. Duggar (1899) has also recorded the occurrence of binucleate pollen mother cells in *Bignonia venusta*. Though there is no difference in size between the binucleate PMC., and the normal one, the nuclei of the former looked different from that of the latter. As can be seen from text figs 17 and 18, the two nuclei of the bi-nucleate PMC., were crescent-shaped, while that of the normal one was quite spherical. The crescent-shaped appearance of the two nuclei may be merely a stage prior to their degeneration.

Besides the occurrence of such bi-nucleate PMC, it is also likely that PMCs., with more than 2 nuclei are formed. Text fig. 19, and photomicro-

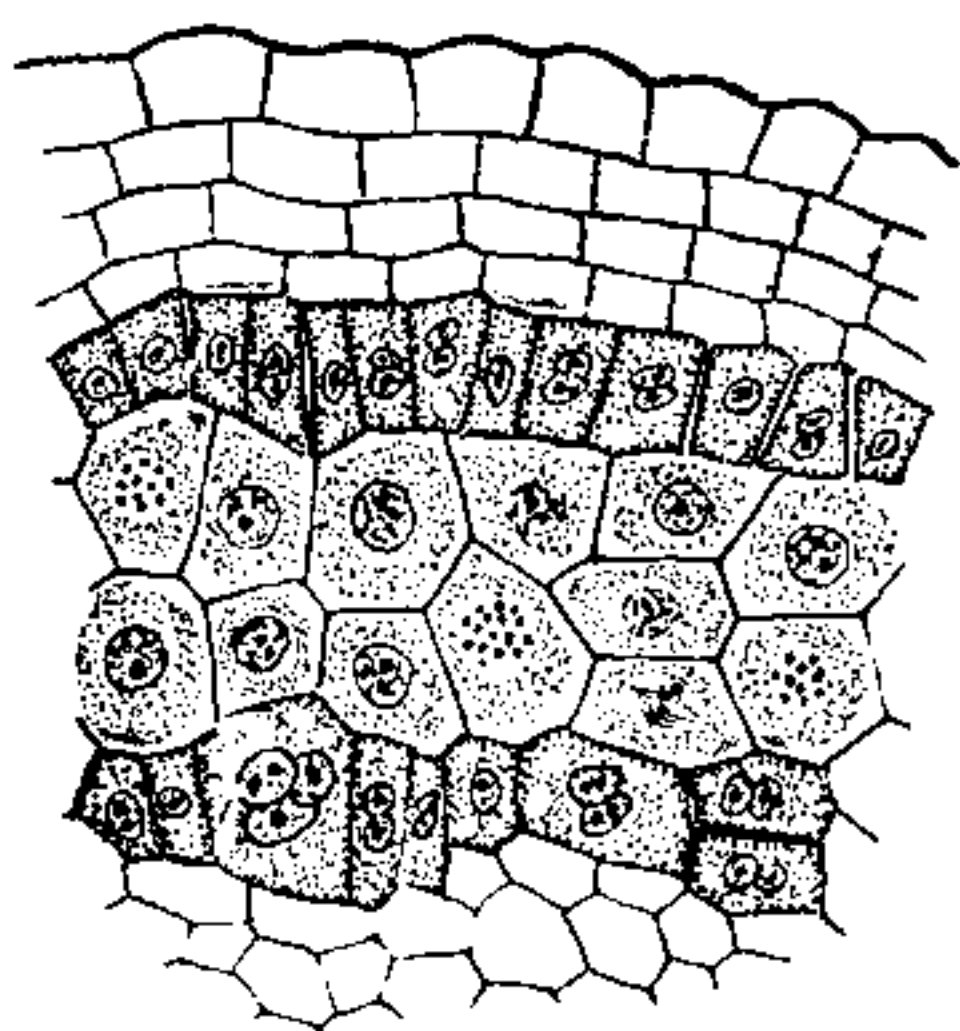
is at least 3 or 4 times as big as the normal PMC. It has to be noted that text figures 17, 18 and 19 have been drawn under the same magnification. Though only one nucleus is seen in the giant PMC., referred to, there is no doubt that it represents a multinucleate PMC. The absence of the other nuclei may be due to the fact that the section was too thin to include these or, they were eliminated during cutting. Considered from the point of view of size, it is likely that this giant PMC., has been formed as a result of fusion of 3 or 4 PMCs., with subsequent dissolution of the partition walls of the individual pollen mother cells. Such giant PMCs., were also found in *Tridax procumbens* as a result of fusion of all the PMCs contained in the locus of an anther (Raghavan and Venkatasubban, 1941). Such giant Pollen Mother cells may give rise to polyploid gametes or as Matsuura (1935a) has pointed out in *Phacellanthus* sp. merely degenerate.

(c) MICROSPORANGIAL TAPETUM

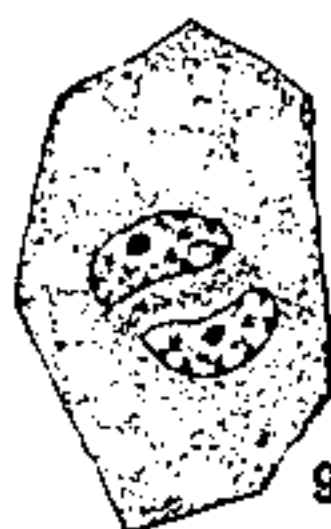
The Tapetum :—The tapetal cells become fully organized, by the time the pollen mother cells are about to enter upon the meiotic divisions. The tapetal cells at this stage were quite conspicuous, and surrounded by a parietal layer, four cells thick (text fig. 98). The wall cells appear to be firmly built, as these were not

Legend to text figures 98-105 (*Tapetum in Crescentia Cujete*)

- Text fig. 98 : Part of the anther locus of *Crescentia Cujete* showing parietal layer and the conspicuous tapetal cells; note that some of the cells are uni-nucleate while others are multinucleate. $\times$ ca 380.
- „ 99 : Tapetal cell at first telophase. $\times$ ca 1900.
- „ 100 : Tapetal cell showing 4 nuclei. $\times$ ca 1900.
- „ 101 : Tapetal cell in which the 4 nuclei in the act of fusing in pairs. $\times$ ca 1900.
- „ 102 : Tapetal cell at telophase after the fusion of the 4 nuclei in pairs. Apparently there are only two nuclei but the presence of 2 nucleoli in each would suggest that these are formed as a result of fusion of 2 nuclei. $\times$ 1900.
- „ 103 : A mature tapetal cell of *Crescentia Cujete*; apparently binucleate but really 4 nucleate. Note the presence of prochromosomes in these. $\times$ 1900.
- „ 104 : A 'Syntapetal cell' likely to have been formed by the fusion of two normal sized tapetal cells $\times$ ca 1900.
- „ 105 : Part of the anther locus showing intrusive tapetum of an 'aggressive nature' $\times$ ca 380.



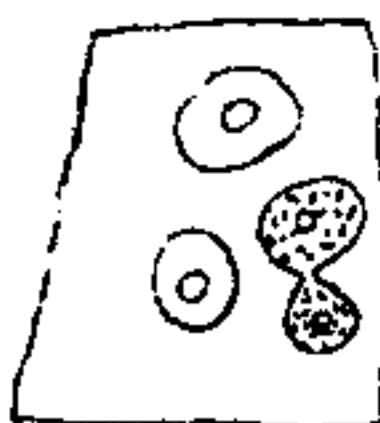
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99



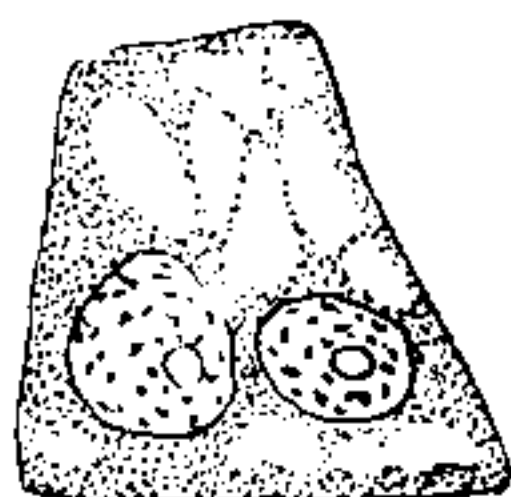
100



101



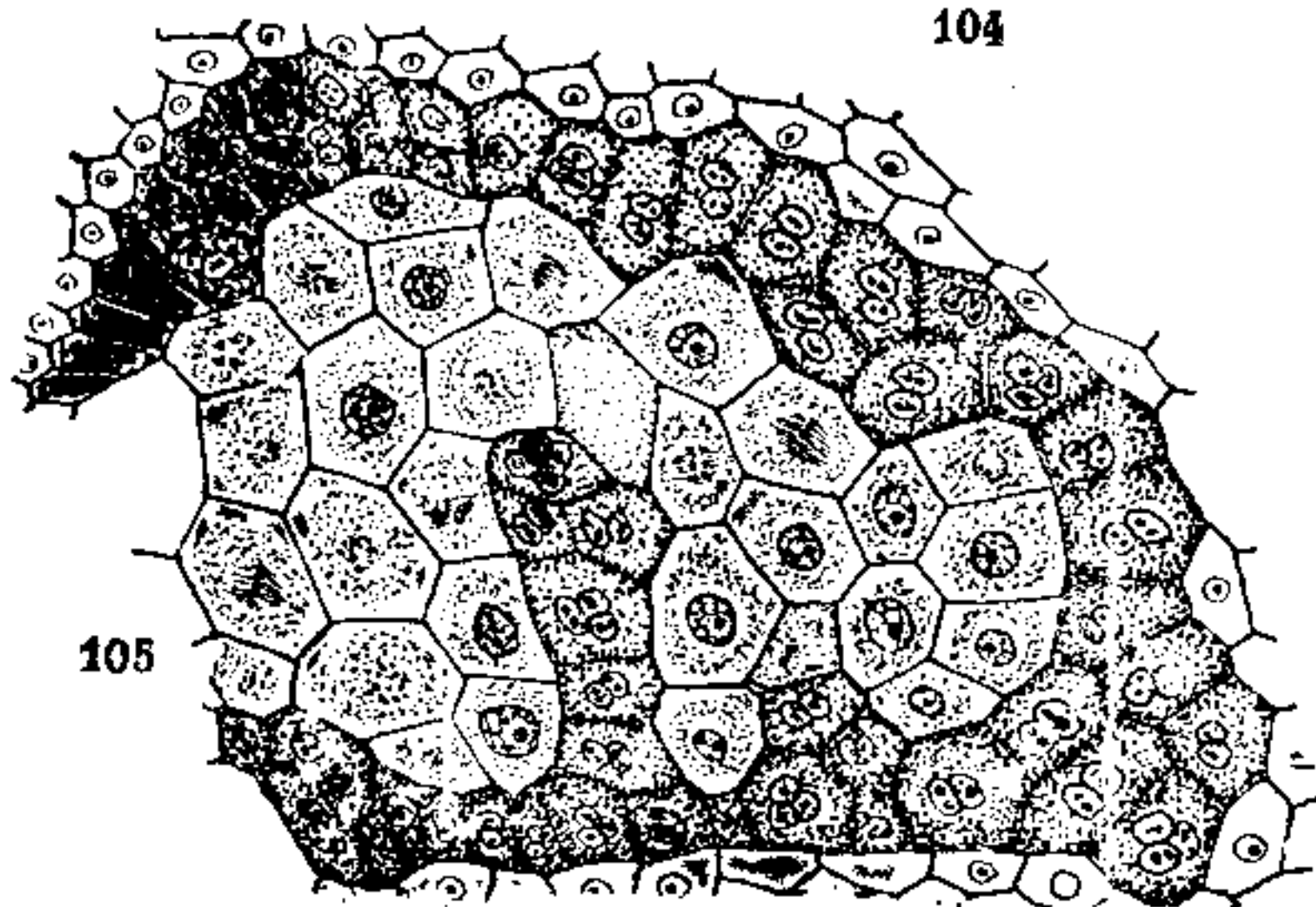
102



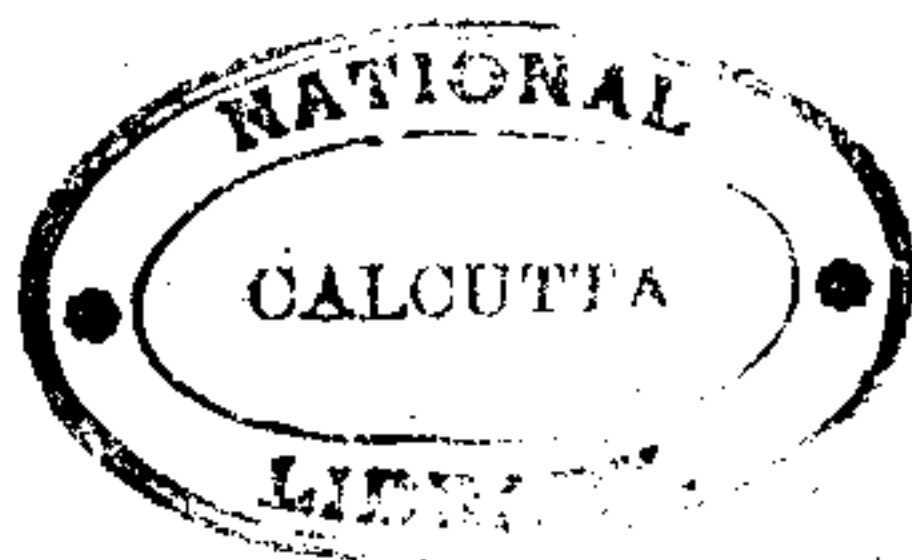
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104



105



crushed, even at the time of pollen grain formation. In this respect, *Crescentia Cujete*, differs from *Stenialoma stans*; for, in the latter, the wall cells and also the tapetal layer get disorganized even before the pollen mother cells enter upon meiosis. The tapetal cells in *Crescentia Cujete*, to begin with, were uninucleate. But owing to rapid division, most of the cells assumed a multinucleate condition. Text fig. 99 shows a tapetal cell at the telophase stage. No wall formation took place and the two nuclei formed as a result of division of the single nucleus originally present in the tapetal cell, once again divided to give rise to 4 nuclei. Text figures 98 and 100 show a few of the tapetal cells containing 4 nuclei. Very soon the 4 nuclei fused in pairs. Text figure 101 shows two of the nuclei in the act of fusion. Text fig. 102 shows apparently only 2 nuclei at telophase; but each one of these contains 2 nucleoli and therefore has been formed as a result of fusion of 2 nuclei. Thus the mature tapetal cell, though apparently binucleate is really 4-nucleate. Prochromosomes were seen in these tapetal nuclei (text fig. 103).

Occasionally, two of the tapetal cells would appear to fuse to form one big tapetal cell. Text figure 104 shows such a tapetal cell. It is nearly twice as big as the normal tapetal cell (compare text figs 104 and 99). The side view of the meta-

phase plate represented in text fig. 104 would unmistakably point out to the increased number of chromosomes in this 'Syn-tapetal-cell.'

A very conspicuous feature of the tapetum lies in its intrusive nature. Intrusive tapetum has been recorded for *Elettaria Cardamomum* (Gregory, 1936), *Gynandropsis pentaphylla* (Raghavan, 1938) and *Alpinia calcarata* (Raghavan, and Venkatasubban, 1940). In all these cases, the intrusion into the anther sac is comparatively superficial, and may be termed 'mild'. But in this case, the tapetum has intruded to such an extent, that it has almost divided the anther loculus into two compartments (text fig. 105) Such an 'aggressive' intrusion of the tapetum, getting right across the anther loculus has also been met with in *Dolichandrone Rheedii* (Unpublished work of Venkatasubban). A similar type of intrusive tapetum has been recorded also for *Lathyrus* (Latter, 1926), *Chisocheton divergens* (Wiger, 1935) and for *Cicer* (Iyengar, K. 1939). Tischler (1927) has observed in *Ribes Gordonianum*, that the entire anther sac was filled with the outgrowth of the tapetum. Recently, Choudhuri (1942), has recorded for two species of *Limonium* (*L. rariflora* and *L. vulgare*), a similar intrusive type of tapetum, which divided the pollen sac into several compartments, and each compartment contained only one pollen mother-cell.

The intrusive tapetum must be regarded as somewhat of a haustorial character. Just as the endosperm haustorium is a means to supplement the somewhat defective function discharged by both the cellular endosperm and the nucellus, the intrusive tapetum may also be regarded as playing a similar role.

There can be no question about the nutritive role of the microsporangial tapetum. The attainment of the pleurinucleate condition, must obviously be taken as an indication of the effort on the part of the tapetal cells, to supply nourishment to the developing pollen mother-cells. The fact that in most cases, the tapetal cells are disorganized after the meiotic divisions of the PMCs., would warrant the above made suggestion, of the nutritive role of the tapetum. But the significance of this deep and aggressive intrusion on the part of the tapetum, is by no means clear. Presumably, the comparatively large anther sac with numerous pollen mother cells, may be responsible for the division of the anther sac into smaller chambers, thus facilitating the free contact and consequent passage of material from the tapetum to the PMCs., Raghavan and V. K. Srinivasan (1942) have discussed at some length, the analogy between the microsporangial tapetum and the nucellar tapetum. They have also indicated the existence of a correlation between the

nucellar tapetum and other associated tissues like nucellus, endosperm, endosperm haustoria etc. It was found that generally speaking, the nucellar tapetum occurred only where, the nutritive role of the nucellus proved defective either by its absence or otherwise. It was only in such cases that endosperm haustoria of some kind or another was met with. This would indicate that the endosperm haustoria is a supplementary measure to restore the defective nutritive mechanism. It was further indicated, that the tapetal layer acted also as a 'liason tissue' helping the transference of nutritive material from the surrounding integumentary tissue.

From what has been said above, the intrusive tapetum can be regarded as haustorial in character. Just as the endosperm haustoria are a means to supplement the somewhat defective nutritive function, discharged by the cellular endosperm and the nucellus, the tapetal intrusion may be regarded as playing a similar role. Whether the tapetal cells act as a intermediary tissue between the PMCs., and the parietal tissue, cannot be said with certainty; for the nutritive role of the latter has not been established.

V. Meiosis in allied genera.

(a) *Millingtonia hortensis* Linn.:—Unlike *Crescentia Cujeete*, the PMCs., at the onset of

meiosis were separate and without any angularities. During diakinesis (text fig. 20) 15 bivalents were observed. These consisted of 2 rings and 13 rods. Out of the few counts made, it may be said that nearly 25% of the bivalents formed rings and the rest rods. This shows that terminalization of chiasmata was almost completed by the beginning of metaphase I. The bivalents were distributed around the periphery and were almost equidistant from one another. According to Catcheside (1937), this spatial arrangement is evolved on account of mutual repulsion. Metaphase I was characterized by the presence of 15 bivalents (text fig. 21). These though appeared free in a few, were mostly found in groups of two's and three's, (text figs. 55-66). In other words, secondary association was in evidence as in *Crescentia Cujete*. Unlike *Crescentia Cujete*, anaphase appeared to be normal and no laggards were observed (text fig. 22). Interphase appeared to be of a very short duration, since none of the preparations revealed this stage. In metaphase II, the 15/15 arrangement was seen (text fig. 23). A few of the plates also exhibited secondary association at this stage. The pollen tetrads were arranged tetrahedrally or isobilaterally and in most cases 6 spindles were observed (text fig. 24). The pollen grains at the time of shedding were found to be in a binucleate con-

(b) *Tabebuia pentaphylla* Hense:— At diakinesis only rod-bivalents were observed (text fig. 26). Text fig. 27 represents the first metaphase, where the 20 bivalents could be counted easily. Secondary association has also been found (text figs 68-82). Text fig. 28 shows metaphase II, where 20 univalents could be counted in each plate. Secondary association in metaphase II could not be made out.

(c) *Parmentiera cerifera* Seem:—Chromosome numbers in the first and second division of meiosis were observed. Text figures 34 and 35, represent the first and second Metaphase respectively. The haploid number for this species has been found to be $n = 20$. First metaphase as in previous cases, was characterized by the presence of secondary association (text figs. 89-91 and Pl. 1, Figs., 14-17)

(d) *Tecoma capensis* Lindl:—Text figures 83-88, show Metaphase I. These not only show the presence of 17 bivalents, but also secondary association. Enough material was not available to make a detailed study, as has been done with reference, to *Crescentia Cujete*, *Tabebuia pentaphylla* and *Millingtonia hortensis*.

(e) *Tecoma stans* Juss:—Twenty bivalents were seen in Metaphase I as shown in text fig. 32. It is likely that this species may

(f) *Stereospermum chelonoides* DC :—A bunch of flowers were obtained from the Agri-Horticultural gardens, Madras. Materials fixed from this bunch, showed only second metaphase and second anaphase. These are illustrated in text figures 31 and 33. In one of the second metaphase plates, 20 univalents were counted. Second anaphase appeared to be irregular, since the univalents were found distributed at random over the spindles, (text fig. 33).

(g & h) *Bignonia megapota mica* Spreng & *Bignonia magnifica* Bull :—The haploid number for *Bignonia megapota mica* has been found to be $n=20$. For *B. magnifica* (text fig. 29) it was found to be the same number. These numbers were determined from metaphase I.

VI. Secondary Association

(a) *Crescentia Cujete* :—For the study of secondary association, this species was found to be quite suitable, as plenty of PMCs., were in a state of division and the bivalents relatively big. Secondary association, as has been pointed out before, was met with in both the first and second metaphase plates. However, observations were made mostly on the first metaphase plates, though a few second metaphase plates were also included. Table 1, shows the various groups of bivalents met with in the first metaphase, while table 2 shows the various associations found in M II. These are illustrated in

text figures 36-54. Some of these have been photographed and are included in Pl. 1, figs. 6-12.

A very characteristic feature that may be seen from the data, is that the bivalents seldom remained in a free condition. Out of a 100 plates which were examined, only one showed the bivalents in an un-associated condition except for one group of two. The observed data fell into 23 classes and the range of variation with reference to the number of association was from 1-10. Another equally characteristic feature is, that association of 2 bivalents were met with more frequently than the rest. In some extreme cases, as many as 9 groups of 2 bivalents and two free bivalents, giving an association of 9 were seen (text fig. 46). However this condition was observed only in two out of about 100 cases. On the other hand, 8 (2) and 4 (1)—an association of 8 was found to the extent of nearly 10% of the total, (text fig. 45 and photomicrograph Pl. 1, fig. 6). Besides such association of 2 bivalents, association of 3 bivalents were also observed in a number of cases. In one case, an association of even 4 bivalents was seen. But one cannot be too sure of this last case, as such an association was observed in only one out of about one hundred.

The different associations, ranging in value from 3 to 10, are represented in text figures 36-49 and also in Pl. 1 figs 6-9. Among the first

Explanation of text figures (33-91)

Text figures 33-91: All the figures have been drawn at an approximate magnification of $\times 3600$, except fig. 33 which has been drawn at a magnification of $\times 1800$.

33. PMC of *Stereospermum Chelonoides* in Second anaphase.
34. PMC of *Parmentiera cerifera* in first metaphase.
35. PMC of *Parmentiera cerifera* in second metaphase.
- 36-49 PMCs of *Crescentia Cujete* in first metaphase showing various degrees of secondary association.
36. 14(1) 3(2) 39. 11(1) 3(2) 1(3)
37. 12(1) 4(2) 40. 8(1) 6(2)
38. 10(1) 5(2)
41. 9(1) 4(2) 1(3); same as microphotograph Pl. 1 fig. 8.
42. 6(1) 7(2) 44. 6(1) 4(2) 2(3)
43. 7(1) 5(2) 1(3)
45. 4(1) 8(2) same as microphotograph Pl. 1. fig. 6.
46. 2(1) 9(2)
47. 5(1) 3(2) 3(3) same as microphotograph Pl. 1. fig. 7.
48. 2(1) 6(2) 2(3) Maximum Association; Microphoto. Pl. 1. fig. 9.
49. 3(1) 4(2) 2(3) * Maximum Association.
50. One of the Second metaphase plates showing an association of 2(1) 6(2) 2(3) Maximum Association; Microphoto Pl. 1. fig. 12.
- 51-54. Secondary association as seen in the second metaphase of *Crescentia Cujete*.
51. One of the second metaphase plates showing an association of 9(1) 4(2) 1(3):

52. 1(1) 8(2) 1(3) & 5(1) 6(2) 1(3)
 53. 7(1) 5(2) 1(3) & 5(1) 6(2) 1(3)
 54. 6(1) 7(2) & 4(1) 5(2) 2(3)

Text figures 55-67 illustrate secondary association in the First metaphase of *Millingtonia hortensis*.

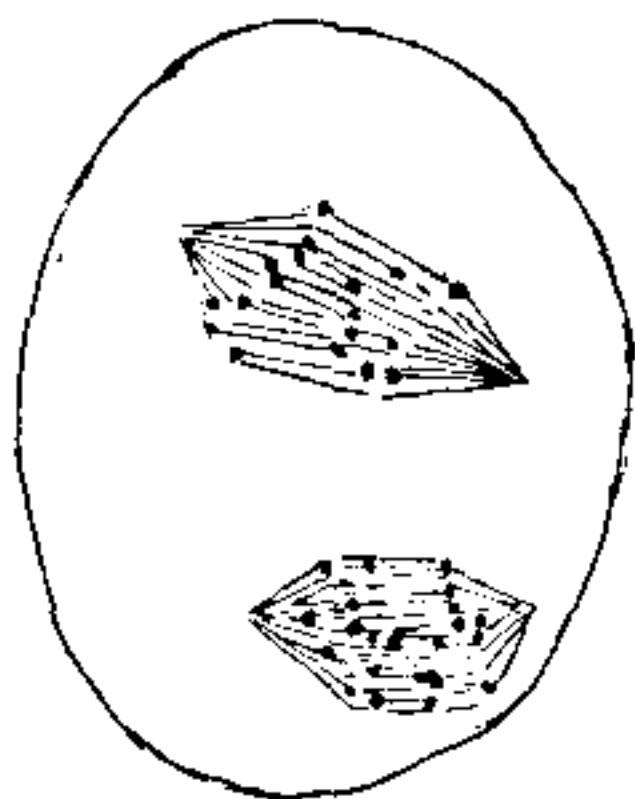
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|---|--------------------|
| 55. 15(1) | 61. 6(1) 3(2) 1(3) |
| 56. 13(1) 1(2) | 62. 5(1) 5(2) |
| 57. 11(1) 2(2) | 63. 7(1) 1(2) 2(3) |
| 58. 10(1) 1(2) 1(3) | 64. 5(1) 3(2) 1(4) |
| 59. 7(1) 4(2) | 65. 5(1) 2(2) 2(3) |
| 60. 8(1) 2(2) 1(3) | 66. 3(1) 6(2) |
| 67. 2(1) 5(2) 1(3) * Maximum association. | |

Text figures 68-82 represent some of the associations met with in the first metaphase of *Tabebuia pentaphylla*.

- | | |
|--|--------------------|
| 68. 14(1) 3(2) | 74. 9(1) 4(2) 1(3) |
| 69. 15(1) 1(2) 1(3) | 75. 5(1) 6(2) 1(3) |
| 70. 12(1) 4(2) | 76. 6(1) 4(2) 2(3) |
| 71. 14(1) 2(3) | 77. 4(1) 8(2) |
| 72. 11(1) 3(2) 2(3) | 78. 6(1) 4(2) 2(3) |
| 73. 10(1) 5(2) | |
| 79. 3(1) 7(2) 1(3) * Ca x 5200. | |
| 80. 3(1) 4(2) 3(3) | |
| 81. 4(1) 2(2) 4(3) Maximum association. | |
| 82. 2(1) 6(2) 2(3) | |
| 83.-88 ; MI. I of <i>Tecoma capensis</i> ; | |
| 83. 13(1) 2(2) | 86. 7(1) 5(2) |
| 84. 10(1) 2(2) 1(3) | 87. 5(1) 6(2) |
| 85. 8(1) 3(2) 1(3) | 88. 7(1) 2(2) 2(3) |

Text figures 89-91 ; MI of *Parmentiera cerifera*.

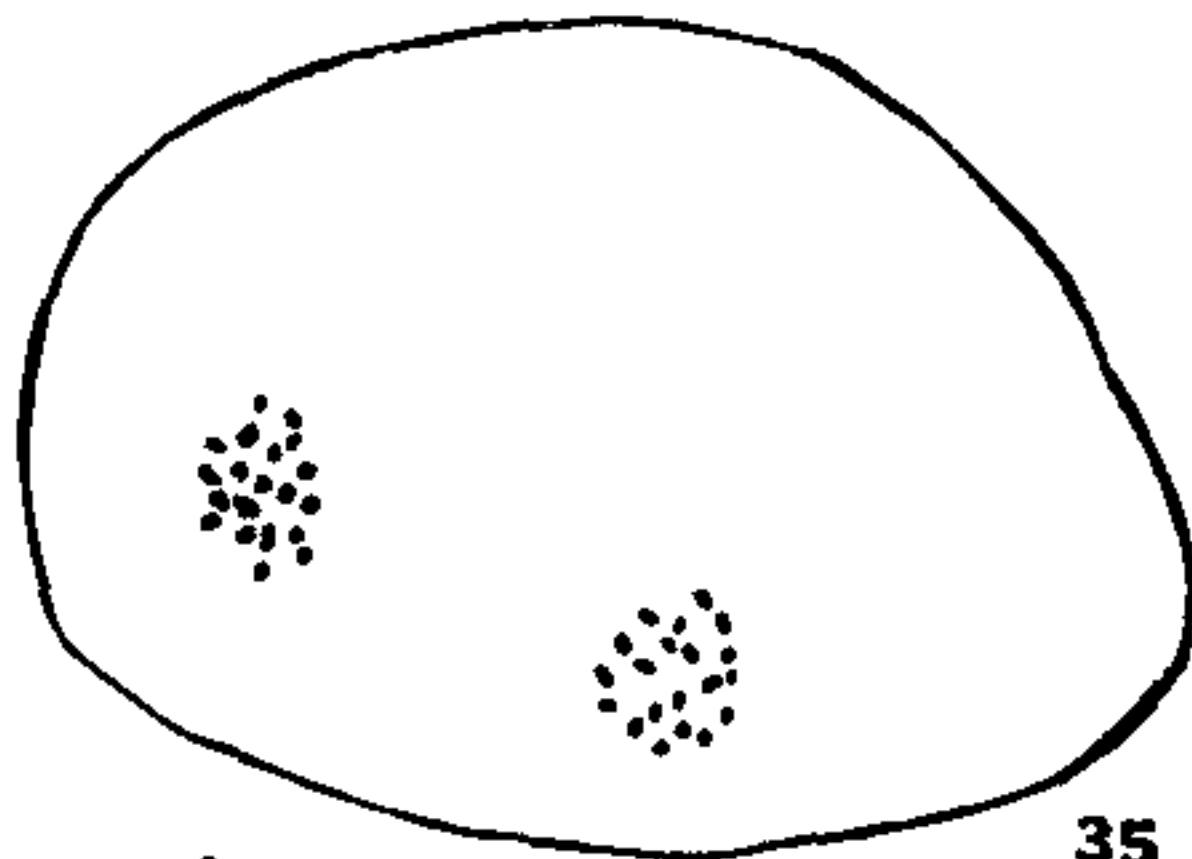
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|---|------------------|
| 89. 7(1) 5(2) 1(3) same as Photomicrograph
Pl. 1. fig. 15. | |
| 90. 5(1) 6(2) 1(3) | „ Pl. 1. fig. 16 |
| 91. 2(1) 6(2) 2(3) | |



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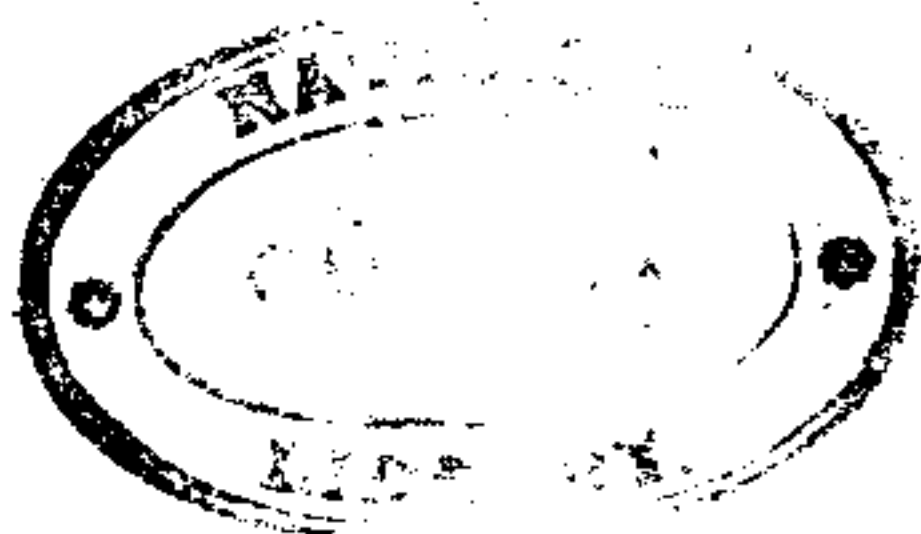


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metaphase plates exhibiting various association, two cases have been singled out for special mention, since they bear a clue regarding the the phylogeny of the species under consideration. In text fig. 49 is represented an association consisting of 3(1) 4(2) and 3(3), while in text fig. 48, is seen an association of 2(1), 6(2) and 2(3). The same is seen in photomicrograph, Pl. 1 fig. 9. Both these types represent the maximum association, as could be judged from the available data. The basic number with reference to the maximum association was found to be 10, since the maximum association consisted of 10 groups. Of the two groupings representing the maximum association, one had a frequency of 9%, while the other was found only to the extent of 2%. Very likely, the former represents the true maximum association, if associations of three bivalents are regarded as natural.

Discrepancies as have been seen from the occurrence of two different combinations, giving the same maximum association, may be due to several causes. For instance, there is the error in judgement or, a slight shrinkage in the cytoplasm will cause adjacent groups of 2 bivalents to appear as 1(3) and 1(1). In other words, there is a certain amount of unavoidable error of a subjective nature, in such studies. Catcheside (1937) has analysed these various sources of error and has put forward important conclu-

sions regarding the nature of secondary association. However such errors can be compensated to some extent by taking a large number of readings, as is generally done in such studies. In this case, it is fairly clear that the combination 3(1), 4(2) and 1(3) represents the maximum association, as the frequency of this was much greater than the other combination which also gave the same value 10, for the maximum association.

Though secondary association has been observed in a number of second metaphase plates also, these were not found suitable for making a detailed study. This may be partly due to the comparatively small size of the univalents. However, a few counts have been made and these are given in table 2. They are illustrated in text figures, 50-54 and photomicrograph Pl. 1 figs, 10-12. While no generalization is possible, since the data available are of a very meagre nature, it can be said that associations of 7, 8, 9 and 10 have been met with, as in the first metaphase. The maximum association of 10 has been observed here also, (text fig. 50, Photomicrograph, Pl. 1 fig 12). A reference to table 2 will show, that one and the same PMC, may exhibit different associations in the two metaphase plates. For example, in text fig. 52 one of the plates revealed an association of 10, that is the maximum association, while the other

exhibited only an association of 8. Again in text fig. 54, one of the plates disclosed an association of 7 while the other revealed the number 9. This would show, that fresh associations are formed during metaphase II and the position taken up by the various univalents at prometaphse II, determine the nature of these.

Table 1, showing secondary association in M I.

(Crescentia Cujete)

No. of Secondary associations.	No. of bivalents in association.				No. of cases.	Total.
	1	2	3	4		
1	18	1	...	...	1	1
2	16	2	...	...	2	2
3	14	3	...	...	3	3
4	12	4	...	...	6	6
5	10	5	...	...	8	9
	12	1	2	...	1	
6	9	4	1	...	6	12
	8	6	...	...	6	
7	8	3	2	...	3	
	7	5	1	...	6	14
	6	7	...	...	5	
8	6	4	2	...	3	
	5	6	1	...	5	19
	7	2	3	...	1	
	4	8	...	...	10	
9	2	9	...	...	2	
	5	3	3	...	6	15
	4	5	2	...	7	
10	3	4	3	...	9	11
	2	6	2	...	2	
	4	3	2	1*	1 aberrant	
						92

Table 2, showing secondary association in M II.
(*Crescentia Cujete*)

No. of secondary associations.	No. of bivalents in association.				
	1	2	3	4	
3	14	3			*other plate in side view.
6	9	4	1	...	
9	4	5	2	...	
7	6	7	...	...	
7	7	5	1	...	
8	5	6	1	...	Other plate not clear
9	4	5	2	...	" "
10	2	6	2		
	1	8	1		

(b) **Secondary Association in *Millingtonia hortensis* Linn.**

The chromosomes were smaller as compared to those of *Crescentia Cujete*. Secondary association has been observed both in the first and second metaphases. These are illustrated in text figs, 55-66 and tabulated (Tables 3 and 4). Secondary pairing may said to be somewhat of a weak nature in this than in *Crescentia Cujete* and *Tabebuia pentaphylla*. This can be inferred from the fact, that out of 55 PMCs., examined, as many as 15 showed the bivalents in a free and unassociated condition. There is also the possibility that this has been resulted from the position effect. The groupings fell into 21 classes; but the last 6 classes have been regarded as aberrant cases. These were regarded so,

on account of their very low frequencies and due to the occurrence of 4 bivalents in association. The absence of 4 bivalents in association, in the related genera showing secondary association, is also in favour of this view.

Similar aberrant cases have been recorded in *Brassica* species by Catcheside (1936). Barring these aberrant cases, there are 16 classes, which may be regarded as normal, exhibiting associations varying from 1-7 and 7 being the maximum association for this species. The maximum association consists of two different groupings as has been met with for *Crescentia Cujete*. In one case, it was resolved into 1(1) and 7(2) while in the other there were found 2(1), 5(2) and 1(3). On the basis of maximum association, this species has a basic number, 8, since either of these combinations representing the maximum association, consisted of 8 groupings.

Table 3, showing Secondary association in *Millingtonia hortensis* in first metaphase

No. of Secondary associations	No. of bivalents in association				No of cases.	Total.
	1	2	3	4		
0	15	...	...	...	8	8
1	13	1	...	...	4	4
2	11	2	...	...	6	6
3	9	3	...	...	3	5

No. of Secondary associations.	No. of bivalents in association				No. of cases.	Total.
	1	2	3	4		
4	8	2	1	...	2	6
	7	4	...	...	4	
5	7	1	2	...	4	12
	6	3	1	...	5	
	5	5	...	...	3	
6	3	6	...	...	1	6
	5	2	2	...	3	
	4	4	1	...	2	
7	2	5	1	...	3	5
	1	7	...	...	2	

* Aberrant cases.

7	3	4	...	1	1	1
7	4	2	1	1	1	1
8	2	2	3	...	1	2
	1	5	...	1	1	
9	1	2	2	1	1	1
						Total 57

**Table 4. showing secondary association in
Millingtonia hortensis in Metaphase II**

0	15	...	...	...	4	4
1	13	1	...	...	2	2
2	11	2	...	...	2	2
3	9	3	...	...	1	1
4	7	4	...	...	2	2
						Total 11

**(c) Secondary association in Tabebuia
pentaphylla Hense :—**

As most of the PMCs., in division were seen only in side view, it was not possible to gather

sufficient data regarding secondary association. However, about 20 plates were seen in polar view and in these secondary association was observed. Text figures 68-82, and photomicrograph Pl. 1 figs 13 & 14 illustrate some of these groupings observed. Table 5, gives the data arranged in the order of association. From the table, it can be seen that the maximum association for this species is also 10 (text figs 79-82 and Photomicrograph Pl. 1. fig. 14). There were three different combinations to give this maximum number. These were 3(1) 4(2) and 3(3); 4(1) 2(2) 4(3) and 2(1) 6(2) 2(3). Similar cases have been met with in *Brassica Sinapis-trum* (Sikka, 1940). He found for the maximum association in this species, two different groupings namely, 1(3) 2(2) and 2(1); and 4(2) 1(1). These he explained as due to some of the chromosomes having undergone structural changes and resulting in the different distribution of homology. The slight variation in the groupings especially with regard to the maximum association, is also likely to be caused when the pairing members happen to lie outside the field of attraction. That structural changes might have taken place in *Tabebuia pentaphylla*, may be inferred from the fact, that during diakinesis all the 20 bivalents formed only rods with a single chiasma. This would mean that the conjugating chromosomes were not identical but

dissimilar to a certain extent. The maximum association for this species has been found to be 10.

Secondary association was in evidence in other related genera also. For instance it was observed in a few cells of *Tecoma capensis*, during metaphase I. Text figures 83-88 represent some of the associations observed. As only very few cells were available for study, it was not possible to say anything regarding the maximum association for this species. In another allied genus *Parmentiera cerifera*, the same phenomenon was found repeating itself. Here also, for want of enough material, sufficient data could not be collected. However it is significant that in the few cells which were examined, the maximum association of 10 was observed twice (text fig. 91). This may be regarded as corroborative evidence to show that 10 is likely to be the basic number for most of the 40-chromosomed species of this family.

**Table 5, showing secondary Association in
Metaphase I in *Tabebuia pentaphylla*.**

No. of Association	No. of bivalents in Assn.			No. of cases	Total
	1	2	3		
0	20	...	...	2	2
3	14	3		1	
	15	1	1	2	4
	14	3		1	
4	14	0	2		4
	12	4			

No. of Association.	No. of bivalents in Assn.			No. of cases	Total
	1	2	3		
5	11	3	1	2	4
	10	5	...	2	
6	9	4	1	3	4
	8	6	...	1	
8	6	4	2	2	
	5	6	1	1	6
	4	8	...	3	
9	3	7	1	1	1
10 Max. Assn.	3	4	3	1	
	4	2	4	1	4
	2	6	2	2	
Total					29

VII. DISCUSSION

(a) **General considerations of the phenomenon of secondary association**.— Secondary association seems to be widespread in its occurrence. More than about 30 of the families both Dicotyledons and Monocotyledons have so far been recorded to exhibit this phenomenon. A classified list of the various families, genera and species, exhibiting secondary association together with their chromosome number, is appended at the end of this paper.

Of the various families showing secondary association, the members of the family *Cruciferae* seem to be more favourable for such a study, as these have been subjected to repeated investigation. The allied family *Capparidaceae* also exhibits this feature amongst its representatives, as has been found by Raghavan (1938)

for *Gynandropsis pentaphylla*, Raghavan and Venkatasubban (1939a) and (1940b) for *Crataeva religiosa* and *Capparis zeylanica*. To these can be added the family Bignoniaceae, where also, several species exhibit secondary pairing as can be seen from the present investigation.

It was in *Oryza sativa*, that this phenomenon was first observed and reported (Kuwada, 1910). This was closely followed by a similar observation on *Dahlia variabilis* by Ishikawa (1911). In spite of these early observations, serious attention was paid to this, only as late as the year 1928, when Darlington, working on *Prunus* recognized two types of pairing, which he designated as prophase pairing and secondary pairing or secondary association. Though some authors like Heilborn (1937) would object to this terminology—Secondary association, it is still in vogue. The first important application of secondary association was made by Darlington and Moffet (1930), for the elucidation of ancestral homology in the *Pomoideae*. From a study of secondary pairing in this group, the authors found that the basic number was 7, from which, the inference was drawn, that by successful amphidiploidy and doubling of the whole genome and more than doubling of a few chromosomes, the secondary basic number 17 was established. According to them it is from this basic number, the existing polyploid forms

Lawrence (1931a) has paid considerable attention to the study of this phenomenon. He found that it is essentially a post synaptic one, which manifests itself as differential approximation of the bivalents in the equatorial plane. From a large number of observations, he emphasized the absence of any pairing or association prior to prometaphase among those bivalents, which are found to be associated at metaphase. In other words, secondary association becomes evident only from the first metaphase onwards. From his studies on *Dahlia* sp., and related forms, Lawrence (1931a) was led to the conclusion, that secondary association is characteristic of allopolyploids, just as multivalent formation is true of autopolyploids. However, it is not true that all allopolyploids exhibit this phenomenon. It is seen only in some of the historically recent allopolyploids.

In any discussion on secondary association, mention has to be made of the work of Gustafsson (1934) and Heilborn (1936). Secondary association as described by these authors, connote something totally different from Lawrence's concept. Gustafsson (1934) found in the EMC., of *Taraxacum*, that the bivalents were brought together at a later stage than diakinesis and they were held in that condition by thread like connections, formed between the conjugating

bivalents. Similar connections have also been noted and figured by Müntzing (1933). Gustafsson did not regard these connections as mere artefacts, but attached considerable significance to these. He interpreted these connections as something quite natural in secondary association.

It has to be pointed out in this connection, that in secondary association, the bivalents are merely approximated and not in actual contact. A number of independent observation on a variety of materials, have established this. Darlington (1937) says "They never contact except through collapse in fixation; they separate regularly into daughter halves without interfering with one another." Lawrence too (1931a) advocated the same view by saying "There will be no material connections and segregation will be unaffected by secondary association". The only instances, where such material connections have been reported are in *Taraxacum* and *Solanum tuberosum* (Müntzing 1933). Matsuura (1935c) too found evidence for the fusion of the chromosome sheaths between the associated bivalents in *Dicentra spectabilis*. The presence of the fibrils among the conjugating bivalents has been explained by Gustafsson as follows :—

"During diakinesis, there is in both sexual and apomictic biotypes, a very high degree of re-

pulsion. During prometaphase, instead of repulsion there prevails a mild attraction and depending on the nature and degree of homology, the bivalents would be lying side by side, lie freely in the vicinity, or may lie so closely that they actually touch one another or may even fuse with one another." According to Gustafsson, the presence of fibril like connections between the conjugating bivalents, not only show that they are highly homologous, but also prove the existence of only terminal affinity among them.

In *Taraxacum*, primary association is suppressed, so that the various groupings seen at a later stage, cannot strictly be regarded as groupings of bivalents. They are only association of univalents. No doubt, secondary association can manifest itself, even among the univalents at first metaphase as has been reported by Catcheside (1934), in the Swede and Turnip groups of *Brassica*, Richharia (1937) in *Raphanus sativus*, *Brassica oleracea* and their hybrids, and by Skovsted (1937) in cotton. Apart from this association prevailing only among the univalents in *Taraxacum*, it appears that the phenomenon exhibited is likely to be something totally different from secondary association. It may be a case of false gemmation. Heilborn (1937) has aptly pointed out that "Gustafsson deals with a quite distinct

phenomenon, viz., a certain kind of late or metaphase conjugation, and not with 'Secondary association' of the common type".

In the paper entitled 'The mechanics of the so-called secondary association between chromosomes,' Heilborn (1936) has given a different interpretation, which does not conform to the generally accepted views on the subject. His conclusions have been based on observations made on a number of species of *Carex*. While in most of the secondary association studies, it is the differential approximation that is taken into account, Heilborn has taken into consideration only the chromosome size. No doubt he has pointed out that the two things, namely, chromosome size and differential approximation refer to two sides of one and the same problem. At the same time he maintained that during the various phases in the meiotic cycle, it is only the spacing of the chromosomes that was affected, while the mutual position of the chromosomes remained more or less the same. This would mean, that the chromosomes or bivalents which are associated during metaphase I or at a later stage, maintained the same relation during prophase and diakinetik stages also. In fact Heilborn is of opinion, that secondary association which is said to commence from prometaphase onwards by several others, existed even in prophase and throughout the

meiotic cycle. But Lawrence (1931a), Catchside (1934, 1937) and others, who have made a careful and extensive search at diakinesis in the several materials exhibiting secondary association, were unable to find any trace of 'differential approximation', among the bivalents, which show the same at a later stage. Further, it is generally believed that secondary pairing is found only in those species having small chromosomes. However, Heilborn found that it manifested in the long-chromosomed species also.

Heilborn's conclusions may be summarised thus :—

(1) Secondary association of chromosomes is due to the action of the forces of nuclear division upon the chromosomes of different size and mass. (2) The greater these differences are, the more pronounced will be the secondary association. (3) Each nuclear division is supposed to be a renewed act of sorting out dissimilar chromosomes and associating the like ones. (4) The preceding nuclear division acts as a centrifuge in sorting out chromosomes of the same size and mass.

If these are the underlying causes of secondary association, then, every plant species should exhibit this phenomenon. For, most of

the plant species exhibit chromosomes of different size and as such are likely to be of different mass also ; repeated nuclear division is also characteristic of most of the plant species. Further, secondary association need not necessarily be found only among the PMCs., during reduction divisions, but they should also prevail when the somatic nucleus divides. No doubt a kind of association called somatic pairing is exhibited by the somatic chromosomes. But opinion is divided, as to whether there is any relationship between this and secondary association.

Somatic pairing is well seen in double nuclei. Such double nuclei according to Huskins and Smith (1932) arise through non-disjunction of the chromosomes at the previous division. Even in ordinary somatic cells, somatic pairing has been found to occur in a number of cases. For instance Raghavan (1935) has observed the same in *Urginea indica*. The same phenomenon has been found in *Dahlia* (Lawrence, 1931b), *Oryza sativa* (Parthasarathy 1938) and in the diploid pollen grains of *Scilla indica* (Raghavan and Venkatasubban, 1939 b).

Davie (1933) has referred to the occurrence of somatic pairing in some of the members belonging to the family Malvaceae. According

to him, somatic pairing need not manifest in the form of paired chromosomes, as is generally the case. The somatic pairing which he has recorded for the Malvaceous genera, consisted of groups of associated chromosomes.

The question as to whether somatic pairing bears any relation to secondary pairing, has been answered in the negative by Darlington (1937). His arguments were, that the rod-shaped nature of the somatic chromosomes and the greater space in which these are distributed, would stand in their way of being associated. On the other hand, Sansome and Philip (1932) have expressed their view, that the two, namely, somatic pairing and secondary association are analogous phenomena. The observations of Skovsted (1933) in Cotton, and of Parthasarathy (1938) in *Oryza sativa*, go to show that plants exhibiting secondary association, may also exhibit somatic pairing.

So far as the present investigation goes, there appears to be no correlation between the two, since somatic pairing was not observed in those cases where, secondary association has been distinctly seen.

VII. (b) Criterion of Maximum Association :—In computing secondary association, it is customary to regard an association of

two bivalents, as representing a single association, an association of 3 bivalents as representing 2, 4 bivalents, as 3 and so on. The basic number for a species exhibiting secondary association is derived from the maximum association. The number of groups found in the maximum association would give the value for the basic number 'b'. If there are six groups as in *Brassica*, then the basic number is 6. In *Gynandropsis pentaphylla* (Raghavan, 1938), a maximum association of 10 was found and since this contained seven groups—1 (1) 2(2) and 4(3), the number 7 was suggested as the basic number for this species.

Kumar and Abraham (1941), have suggested that 'the most frequent type of secondary association should be taken as representing the maximum association'. They quote Catcheside (1937), in support of their suggestion. It may be pointed out that, Catcheside has made a critical study of the problem and from a large number of observations, he arrived at the conclusion, that chance plays an important role and that secondary pairing depends mostly upon the relative position of the bivalents during diakinesis. Therefore it happens that all bivalents which lie adjacent during diakinesis and which are capable of secondary pairing, are so paired at metaphase I. From Catcheside's paper, there is nothing to warrant the assumption that the

was guided by the highest frequency in the determination of maximum association. If that were so, he should have regarded 7 as the basic number instead of 6; for, seven was the modal association. Catcheside has however, made a distinction between normal and aberrant cases. Normal cases were distinguished from the aberrant ones, by the higher frequency of the former and the highest association among the normal ones, has been regarded as representing the maximum association, even though this may not be the modal association.

In the present case, Catcheside's method has been adopted. Out of what appeared to be normal cases, the highest association has been chosen for the maximum association irrespective, of its frequency.

VII. (c) Secondary Association with Reference to Basic Number and Ancestral Homology :— From the data presented in Table 1, it can be seen, that the maximum association value for *Crescentia Cujete* is likely to be 10. The same value for the maximum association has also been revealed for *Tabebuia pentaphylla*. The same is true for another member of the family Bignoniaceae, namely, *Parmentiera cerifera*. All these different species, have also been found to exhibit the same diploid chromosome number, $2n = 40$.

Therefore it is very likely, that 10 represents the basic number for these. The fact that, the majority of the investigated species in this family possess a diploid chromosome number of $2n=40$, gives additional support to this assumption.

A reference to Table 1, will also show, that there are three different combinations, to give the maximum association value, 10. These are, 3(1) 4(2) and 3(3); 2(1) 6(2) and 2(3) and 4(1) 3(2) 2(3) and 1(4). Out of these, the maximum association consisting of the grouping, 3(1) 4(2) and 3(3), has a higher frequency, namely 9% of the total while, the other two have been found to the extent of only 2% and 1% respectively. On the basis of this higher frequency, 3(1) 4(2) and 3(3) has been interpreted as representing the true maximum association. From this, it will follow, that the ancestral form of *Crescentia Cujete* should have had the following constitution:—

AAA

BBB

CCC

DD

EE

FF

GG

H

I

J

Assuming that the 40-chromosomed type as having been derived from a 10-chromosomed ancestor, this may be imagined to have taken place as follows :—If a natural cross had taken place between two such 10-chromosomed species, in one of which, the entire genome had been altered due to gene mutation, the resulting form would have had the following chromosome make up ;—

AA'
BB'
CC'
DD'
EE'
FF'
GG'
HH'
II'
JJ'

Such an offspring would have been highly sterile, as in the cross between *Nicotiana Bigelovi* $\times$ *N. suaveolens* (Goodspeed & Clausen, 1927). However, if there had taken place doubling of the chromosomes either somatically as in *Primula Kewensis* (Newton and Pellew, 1929), or gametically as in *Raphano-Brassica* (Karpechenko, 1927, 1929), an allotetraploid with a high degree of fertility, might have been evolved. The restoration to the fertile condition in this case, would have been due to autosyndesis.

If such a thing had taken place in the evolution of *Crescentia Cujete*, it should have the following genome ;—

AA A' A'
BB B' B'
CC C' C'
DD D' D'
EE E' E'
FF F' F'
GG G' G'
HH H' H'
II I' I'
JJ J' J'

In a form like this, only the chromosomes AA or A' A'—BB B' B', etc., will pair during diakinesis. The chromosomes AA', BB'—JJ' will not be structurally similar and therefore will not form chiasmata during diakinesis; on the other hand, their homology will manifest in the form of secondary association.

From what has been said previously, it may be expected, that there should be 10 groups of two bivalents each, to represent the maximum association. This theoretical figure, was only approached in just a few cases, where groups of 9 two's and 2 free bivalents were actually found (text fig. 46). On the other hand, nearly 10% of the total number of PMCs., examined, exhibited the association, 4(1) and 8(2) (Text fig. 45 and photomicrograph Pl. 1 fig. 6). The fact that association of two bivalents were

more frequently seen than other associations, would suggest, that the real maximum association should consist of 10 groups of 2 bivalents each. The reason for not seeing this grouping, may be on account of the fact that, chance plays a great part in such associations. Further, it should be remembered that, unless the secondary pairing chromosomes happen to occupy a position, quite close to each other during diakinesis, the true maximum combination cannot be observed.

From the observations recorded in table 1, it may be seen that there are, sufficient number of cases where, as many as 3 bivalents were grouped together. The frequency of such cases, warrant the assumption, that these are not aberrant types. Groupings of more than 2 bivalents generally involve structural changes, in the nature of segmental interchange. Thus in the form having the following genome, namely,

AA	A ' A'
BB	B ' B'
CC	C ' C'
DD	D ' D'
EE	E ' E'
FF	F ' F'
GG	G ' G'
HH	H ' H'
II	I ' I'
II	I ' I'

if segmental interchange had taken place between say AA and FF, BB and GG and CC and HH, the resulting form would have undergone the following changes in its chromosome set:—

A(AF)	A ' A'
B(BG)	B ' B'
C(CH)	C ' C'
DD	D ' D'
EE	E ' E'
F(FA)	F ' F'
G(GB)	G ' G'
H(HC)	H ' H'
II	I ' I'
JJ	J ' J'

In this form, a new homology is set up, on account of which, A(AF) F(FA); B(BG) G(GB); C(CH) H(HC), are likely to exhibit pairing. This is due to their possession of common segments. At the same time, their affinity for AA' or BB' etc., is not likely to be impaired in any way. In such cases, the species under consideration is likely to exhibit 3(3) 4(2) and 3(1). Such a combination has been actually observed, in 9 out of about 100 cases. From this, it would become evident, that in the evolution of this species, structural changes too, might have played a part. The occurrence of mostly rod-bivalents during diakinesis, would also point out to the weak

However, there is also the other possibility, namely, that conjugation may be determined by genetic factors, as has been found in *Drosophila* (Gowen 1928) and in *Zea Mays* by Beadle (1930). Sapehin (1933) regarded non-pairing and other abnormalities in reduction division, to be due to the response of a particular genotype to the environment. According to Katayama (1931), Heilborn (1930) and Sax (1931), external conditions like temperature and other factors would affect conjugation. Meurman (1928) has pointed out, that the nutritional conditions of the cell, would also affect the pairing of chromosomes, during diakinesis.

Tabebuia pentaphylla too, has 10 for its basic number. The occurrence of 3 bivalents in association would suggest, that structural changes have taken place in this species also. Through amphidiploidy and structural changes in the chromosomes, the 40-chromosomed species, *Tabebuia pentaphylla* might have been evolved.

In *Millingtonia hortensis*, the maximum association has been found to be 7. There have been found two groupings giving the same value. These were 1(1) and 7(2) and 2(1) 5(2) and 1(3). In both these, there were found only 8 groups and therefore the basic number for

hortensis, having the diploid number, $2n=30$, might represent an allotetraploid, from the basic number 8, in which two of the chromosomes might have been lost. It is also likely that structural changes might have taken place and this is inferred from the occurrence of more than 2 bivalents in association.

Several metaphase plates were examined critically with a view to find whether any ring formation is present; for, structural change of the type of segmental interchange, in most cases, manifests itself in the form of rings. However, no ring formation was found in this or in the allied species. Perhaps in such small-chromosomed species, the segment involved is of a very small order that ring formation may not be visible. Segmental interchange in such cases could only be inferred from the occurrence of three or more bivalents in association or the association of bivalents of dissimilar size. Catcheside (1937) has found such associations between dissimilar bivalents in *Brassica* species. The same has been observed by Sikka (1940) in *Brassica* species, and Raghavan and Venkatasubban, (1940, b) in *Capparis zeylanica*. Such associations are interpreted on the basis of segmental interchange. It may also be mentioned in this connection, that segmental interchange need not always result in ring formation. In support of this may be cited the article

by Clarke and Anderson (1935). In a species of Maize, the authors observed a case of reciprocal translocation without the accompaniment of rings.

VII. (d) **Secondary association and ploidy of the species** :—Secondary association, in the words of Lawrence (1931a) is ‘intimately connected with allopolyploidy.’ He also adds that only recently originated allopolyploids will exhibit this phenomenon, as also a few autopolyploids. In the case of autopolyploids, secondary association will be seen only, if the chromosomes fail to form multivalents. According to Müntzing (1933), secondary association is a rough indication of the age of the species. Though many allopolyploids show this phenomenon more than any others, it should be remembered that there exist allopolyploids of all gradations.

For instance, in the diploid hybrid of a cross between *Crepis rubra* and *Crepis foetida* (Poole 1931), it has been found that there was complete pairing between the chromosomes derived from the parents, as though they had identical chromosome sets. More than this, the tetraploid derived from this hybrid, showed the characteristics of an autotetraploid and as many as 5 quadrivalents were observed.

In the case of the hybrid *Primula Kewensis*, which was evolved as a result of crossing *Primula floribunda* and *P. verticillata* (Newton and Pellow, 1929), the chromosome sets from the two parents paired completely as in the cross between *Crepis fœtida* and *C. rubra*. However, the tetraploid derived from this hybrid was found to be different. In this, there were found a few multivalents and pairing took place only between *verticillata* and *verticillata* chromosomes; so also the *P. floribunda* chromosomes exhibited conjugation.

As a third type, may be mentioned the classical intergeneric cross—*Raphano-Brassica* by Karpechenko (1927). Here, the diploid hybrid as a result of the cross between *Raphanus sativus* and *Brassica oleracea*, exhibited no pairing at all. In the tetraploid form, the pairing was found to be quite simple, regular and complete. In such cases secondary association will be of some use in determining the homology of the chromosomes. In fact, in the absence of any multivalent formation, one has to rely solely on secondary association for the elucidation of chromosome homology. The homologues from the two parents, while they are sufficiently differentiated, not to effect any primary association, will still express their affinity by exhibiting secondary pairing.

With reference to plants like *Primula Kewensis* and *Raphano-Brassica*, which breed true and are highly fertile, their ploidy could only be inferred from such a criterion as secondary association. This is especially true of those polyploids, which have become functional diploids. While numerical consideration alone is sufficient for the detection of polyploidy as in *Salix* (Blackburn and Harrison 1924), or species of *Triticum*, *Chrysanthemum* etc., there are a number of cases where, this is not possible due to the absence of species with a lower basic number, or due to want of those with numbers in multiple relation. In such cases also, secondary association may come in handy in the determination of the nature of the species. Again, for instance in *Pyrus* and allied plants, the lowest number observed is 17 and multiples thereof, (Darlington and Moffet, 1930). But this was found to be too high to represent the basic number of the family. In the meiosis of these species, the authors (Darlington & Moffet) found secondary association and from which the basic number of the family was found to be 7. This led the authors to postulate, that the *Pyrus* group has been derived from a 7-chromosomed ancestor by the duplication of the entire chromosome complement and a further duplication of a few of the resultant chromosomes. In this manner a new or secon-

dary basic number was formed and from which a successful polyploid series was established. Similarly, *Brassica species* ($2n=18$) has been derived from the basic number 6 (Catcheside 1937). *Oryza sativa* is essentially, a diploid in its behaviour. However, secondary association findings in this species has revealed that it is really a secondarily balanced polyploid (Nandi, 1936).

In a few cases like *Tricyrtis latifolia*, *Dicentra spectabilis* and *Acer platanoides*, the true nature of the species concerned could only be made out from secondary association studies. According to Matsuura (1935c), *Tricyrtis latifolia* has a haploid number, $n=13$ and *Dicentra spectabilis* has, $n=8$. In the first meiotic division of both these, two bivalents were regularly approximated, and from this, the author has drawn the inference that the two species are not true diploids, but are only secondarily balanced diploids, from basic numbers 12 and 7 respectively. In a similar way, Meurman (1933) found that *Acer platanoides* is a secondarily balanced species from the basic number 12. Even this basic number, according to him, is likely to be a balanced one in its turn.

In the present case, two basic number have been met with. For *Millingtonia hortensis* ($n=15$), the basic number was found to be 8

while for *Crescentia Cujete*, ($n=20$) and *Tabebuia pentaphylla* ($n=20$), it was found to be 10. Considering the fact that most of the species in this family exhibit a haploid number, $n=20$, a multiple of 10, it is highly probable that 10 is the primary basic number.

Corroborative evidence for the tetraploid nature of the $2n=40$ and $2n=30$ chromosomed species, was obtained through a study of the nucleolar behaviour in *Crescentia Cujete*, *Kigelia pinnata* and *Oroxylum indicum*. In *Crescentia Cujete* 2 nucleoli were observed in the first telophase of the reduction division. In *Kigelia pinnata* 2 bivalents were found attached to the nucleolus during diakinesis. On the basis of evidences presented by de Mol (1927) and Heitz (1931), the two species mentioned above should be in the nature of tetraploids. This would mean that these have been derived from 10-chromosomed ancestors. The same conclusion has also been reached from a study of secondary association in one of these species. In *Oroxylum indicum*, in the somatic mitosis, it has been observed that 4 prochromosomes were attached to the nucleolus. Further, it was also found that during the telophase, there appeared 4 nucleoli. These observations would go to show that *Oroxylum indicum* having a diploid number $2n=30$ is also a tetraploid.

Millingtonia hortensis has also the same chromosome number as *Oroxylum indicum*, ($2n=30$). Since *Oroxylum indicum*, has been found to be a tetraploid, the same may be true of *Millingtonia hortensis* too. The basic number for *Millingtonia hortensis*, from secondary association studies, has been found to be 8. From this it follows, that the somatic chromosome number for this species can be represented as $2n=30=4x-2$.

Regarding the basic number for this family, the numbers 10 and 8 have been suggested already, on the basis of maximum secondary association. However, from the list of chromosome numbers available for the different members of this family, it will be seen that, the lowest number recorded so far is $n=9$. This haploid number $n=9$ has been found for two species of *Incarvillea*. Since this is the lowest number found, it can also be regarded as another basic number. Therefore, the family exhibits the basic numbers 8, 9 and 10, of which, 10 may be regarded as primary, since the majority of the species investigated revealed a diploid chromosome number of $2n=40$.

The question is, how far a higher basic number, in this case 10, can be regarded as primary basic number, especially when basic numbers of a lesser value (8 and 9) have been

found in the same family. There are indeed a few cases, in which the higher number has been selected as the primary basic number, in the face of lower basic numbers. For example, in *Crepis*, the number 5 has been regarded as the primary basic number, even though there are lower numbers than this (Babcock, 1934). That 10 may represent the primary basic number for the family Bignoniaceae, is suggested as a mere possibility. It is not however based on any studies on chromosome morphology, for which the present materials would not lend themselves.

There is also the point, which has to be explained namely, whether a family can have several basic numbers or one only. There are a number of families with more than one basic number. For instance, the family Scrophulariaceae shows a basic number of 6, 7, 9 etc., Fagerlind (1937) has found the basic numbers, 9, 10, 11, 12, 14 and 17 for the family Rubiaceae. There are other instances too, to show the occurrence of more than one basic number, not only in a family but also, in one and the same genus. The genus *Crepis* affords a good example for this. Rosenberg (1918) has given, 3, 4, 5, 6, 8, 10 etc., as the basic numbers for this genus.

Therefore, it should not be surprising, that three basic numbers have been met with among

the different members of the Bignoniaceae. An exhaustive study of the different members of this family, may perhaps throw more light on this question. If 10 is accepted as the primary basic number for this family, the secondary numbers, 9 and 8 could be derived by a process of fusion of some of the chromosomes. Another likely process by which, the secondary basic numbers could be derived, would be through reciprocal translocations between non-homologous chromosomes, followed by meiotic irregularities, which would effect the elimination of two of the chromosomes. The latter process is more plausible so far as the different members of this family are concerned, since secondary association studies have led to the inference, that structural changes, especially of the type of segmental interchange, have played a prominent role in the evolution of the different species.

It is also likely, that the forms which were evolved from the secondary basic numbers, 8 and 9, were not as successful as those evolved from the primary basic number. It is assumed that all the 40-chromosomed species or those having chromosome numbers which are multiples of 40, have been derived from the basic number 10. Out of the 42 species for which the chromosome number is now known, as

many as 27, exhibit the number 40 or multiples thereof.

Secondary basic numbers may sometimes prove very successful as in Pomoideae (Darlington and Moffet, 1930). Under natural conditions, there appears to be a tendency towards the establishment of such secondary basic numbers. Such numbers could be established, either by fragmentation, fusion and reduplication of some of the chromosomes, as also through hybridization. In Kalanchoe, the basic number 15, found in a few species, has been explained, as due to hybridization of forms with basic numbers 8 and 7, (Baldwin, 1938).

VII. (e) Limitations of Secondary Association as a Guide to Ancestral Homology :—Evidence from secondary association cannot be regarded as in itself conclusive, with regard to ancestral homology and basic numbers, unless corroborated by genetical and other evidences. In other words, secondary association has its own limitations. These have been discussed at some length in the paper on the cytology of *Capparis zeylanica* (Raghavan and Venkatasubban, 1941). For one thing, secondary association is found only in recently evolved allopolyploids, while the historically old ones, will behave as true diploids, without exhibiting any

reference to such new forms, that the secondary association method of finding out homology can be applied.

Taking it for granted that observations are made only on well fixed materials, secondary association may be imposed by other conditions such as, structural changes, fragmentation, translocation etc. Such changes in the chromosomes occur under natural conditions, especially in polyploids and in such cases, secondary association results, may likely to produce only a modified value; for, on account of segmental interchange, chromosomes which would normally possess a certain degree of affinity, may become non-homologous. It can also be the other way. Due to segmental interchange, chromosomes which might not have had even distant homology, may get common segments, which would set up a new homology. The presence of too many chromosomes, the element of chance, etc., may also influence the nature of the association.

Further, just as primary pairing is controlled by genetic and environmental factors, secondary association may also have controlling genes, which would operate only under certain conditions. External factors too, may have a more pronounced effect on this phenomenon than has been thought of hitherto.

may be made in this connection, regarding the observations of Tokumoto (1940) on a tetraploid species of *Veratrum oxysepatum*. This species while exhibiting secondary association under normal conditions, revealed the same to a marked extent, when the plant was exposed to low temperature conditions for a while.

Heilborn (1936), regarded secondary association of chromosomes, as resulting from the action of forces involved in nuclear division, and upon the chromosomes of different mass. The greater these mass differences in the chromosomes are, the more pronounced will be the secondary association. According to him all the chromosomes which are derived from nuclear division should exhibit this phenomenon. Heilborn would not attach much phylogenetic significance to this manifestation of the bivalents, nor would he regard the secondarily paired chromosomes, as bearing any ancestral homology.

In spite of these superimposed conditions, which tend to affect the value of secondary association, there are a number of cases on record where, secondary association was not only a regular feature but also, the results obtained from such a study, tallied with those of genetical analysis. The polyploid nature of the *Brassica* species, inferred from

secondary association studies, has been amply supported from the genetical side, by the finding of the presence of duplicate factors for this species (Pease, 1926).

In the present study, structural changes have been assumed throughout, since in most of the species, three bivalents were found in association. Therefore, the question arises, as to how far the results obtained through secondary association studies could be relied upon, since structural changes generally tend to distort the original homology. It is a strange coincidence that the 3 allied genera, *Crescentia Cujete*, *Tabebuia pentaphylla*, and *Parmentiera cerifera*, all having a diploid number of $2n=40$, should exhibit the same basic number 10. The repeated occurrence of this number 10, cannot therefore be ignored. Very likely, the nature of the structural change is only of a mild type, so as not to impair the original homology. The absence of ring formation in these species would to a certain extent help this view.

To sum up, the different species included in the present investigation, were found to be mostly in the nature of tetraploids. Evidence for this conclusion was adduced from three different sources, namely (1) the prevalence of high chromosome number; (2) the occurrence of secondary association and (3) nucleolar

behaviour. Only further work, especially of a genetical nature, would show how far these cytological inferences, drawn in the present studies, are tenable.

VIII. SUMMARY

The haploid chromosome numbers have been determined for the first time for the following plant species :—

<i>Crescentia Cujete</i>	$n = 20$
<i>Millingtonia hortensis</i>	$n = 15$
<i>Tabebuia pentaphylla</i>	$n = 20$
<i>Parmentiera cerifera</i>	$n = 20$
<i>Bignonia rosea</i>	$n = 20$
<i>Bignonia megapotamica</i>	$n = 20$
<i>Tecoma stans</i>	$n = 20$
<i>Tecoma capensis</i>	$n = 17$
<i>Stereospermum chelonoides</i>	$n = 20$

Meiosis has been described in detail in *Crescentia Cujete*, *Millingtonia hortensis* and in *Tabebuia pentaphylla*.

Secondary association has been met with in *Crescentia Cujete*, *Tabebuia pentaphylla* and *Millingtonia hortensis*. The basic number for the first two 40-chromosomed species, was found to be 10, while for *Millingtonia hortensis*, it was found to be 8.

From all available cytological data, it was found, that the family has 3 basic numbers —8, 9 and 10 of which, 10 has been suggested as the primary basic number.

Evidence has been adduced to show that both the 40-chromosomed, and the 30-chromosomed species, are allopolyploids and that structural changes of the chromosomes have played a significant part, in the evolution of these.

The phenomenon of secondary association and its limitations as a guide in the determination of ancestral homology and basic numbers have been discussed at some length.

A classified list of plant genera and species showing secondary association, together with their chromosome numbers, has been included as appendix.

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X. APPENDIX.

A Classified List of Plant Species where Secondary Association has been recorded.

Natural Order.	Genus and Species.	Chromosome Number.	Author, Year etc.
POLYPETALAE :—			
Fumariaceae	<i>Dicentra spectabilis</i>	n = 8	Matsuura, 1935
	<i>Dicentra pusilla</i>	n = 8	Matsuura & Suto, 1935
Cruciferae	<i>Arabis iwatensis</i>	n = 16	„ „ „
	<i>Sinapis alba</i>	n = 12	Haga
	<i>Sinapis dissecta</i>	n = 12	Haga
	<i>Sinapis arvensis</i>	n = 9	Haga
	<i>Cochlearia</i>		
	<i>oblongifolia</i>	n = 7	Matsuura & Suto, 1935
	<i>Eruca sativa</i>	n = 11	Alam 1936
	<i>Barbarea patens</i>	n = 8	Matsuura & Suto, 1935
	<i>Barbarea hondoense</i>	n = 8	„ „ „
	<i>Cardamine pratensis</i>	n = 15	Lawrence, 1931
	<i>Diplotaxis erucaoides</i>	n = 7	Haga, 1938
	<i>Raphanus sativus</i>	n = 19	Richharia, 1937
	<i>Brassica Napus</i>	n = 18	Catcheside, 1934
	<i>Brassica Rapa</i>	n = 10	„ „
	<i>Brassica nigra</i>	n = 8	Haga, 1938
	<i>Brassica oleracea</i>	n = 9	„ „
	<i>Brassica juncea</i>	n = 18	„ „
	<i>Brassica campestris</i>	n = 10	Alam, 1936
	<i>Brassica trilocularis</i>	n = 10	„ „
	<i>Brassica chinensis</i>	n = 10	Richharia, 1937
	<i>Brassica monensis</i>	n = 12	Sikka, 1940
	<i>Brassica sinapistrum</i>	n = 9	„ „
	<i>Brassica pekinensis</i>	n = 10	Richharia, 1937
	<i>Raphanus sativus</i> × } <i>Brassica oleracea</i> }		
	F. & F. Hybrid	n = 18	

Natural Order.	Genus and Species.	Chromosome Number.	Author, Year etc.
	<i>Brassica</i> × <i>pekinensis</i> <i>Brassica</i> × <i>Rapa</i>	n = 9, 11	Richharia, 1937 Secondary association among the univalents in second metaphase.
Capparidaceae	<i>Gynandropsis penta-</i> <i>phylla</i>	n = 17	Raghavan, 1938
	<i>Crataeva religiosa</i>	n = 13	Raghavan & Venkatasubban, 1939
	<i>Capparis zeylanica</i>	n = 17	Raghavan & Venkatasubban, 1941
Malvaceae	<i>Gossypium herbaceum</i>	n = 18	Davie, 1933
	<i>Gossypium aridum</i>	n = 13	Skovsted, 1937
	<i>Malva sylvestris</i>	n = 21	Davie, 1933
	<i>Hibiscus trionum</i>	n = 14	Rao, L.N., 1941
Geraniaceae	<i>Geranium albanum</i>	n = 14	Warburg, 1938
	<i>Geranium asphode-</i> <i>loides</i>	n = 14	" "
Vitaceae	<i>Vitis</i> sp.,		Lawrence, 1931
Sapindaceae	<i>Acer platanoides</i>	n = 13	Meurman, 1933
	<i>Acer Miyabei</i>	n = 13	Takizawa,
	<i>Acer mono</i> var. <i>eupictum</i>	n = 13	"
	<i>Acer Mayrii</i>	n = 13	"
	<i>Acer japonicum</i> var. <i>typicum</i>	n = 13	"
	<i>Acer ornatum</i> var. <i>Matsumurae</i>	n = 13	"
	<i>Aesculus flava</i>	n = 20	Upcott, 1936
	<i>Aesculus Hippocasta-</i> <i>rum</i>	n = 20	" "
	<i>Aesculus pavia</i>	n = 20	" "
	<i>Aesculus carnea</i>	n = 40	" "
Papilionatae	<i>Amorpha fruticosa</i>	n = 19	Matsuura & Suto, 1939
	<i>Cicer arietinum</i>	n = 8	Iyengar, N.K., 1939
Rosaceae	<i>Prunus cerasus</i>	4x = 32	Darlington, 1928
	<i>Prunus Laurocer-</i> <i>asus</i>	22x = 176	Meurman, 1929

Natural Order.	Genus and Species.	Chromosome Number.	Author, Year etc.
	<i>Pyrus Malus</i>	$4x + 6 = 34$	Darlington & Moffet 1930
	<i>Pyrus domestica</i> ×		
	<i>Pyrus cerasifera</i>	$4x = 32$	Darlington, 1930
	<i>Pyrus minima</i>		
	(Triploid) $2n = 51$		Moffet, 1931
	<i>Rubus</i> -‘Loganberry’	$6x = 42$	Crane & Darlington, 1927
	<i>Crataegus</i> sp., <i>Mespilus</i> , <i>Cotoneaster</i> etc.,		
		$4x + 6 = 34$	Moffet, 1931, 1934
	<i>Cydonia cathayensis</i>	$2n = 17$	Moffet,
Saxifragaceae	<i>Tiarella polyphylla</i>	$n = 9$	Matsuura & Suto, 1935
	<i>Hydrangea paniculata</i>		
	var. <i>praecox</i>	$n = 18$	„ „ „
Crassulaceae	<i>Sedum Rhodiola</i> , var.		
	<i>elongatum</i>	$n = 11$	Toyohuku } Cited from
	<i>Sedum yezoense</i>	$n = 11$	„ } Matsuura,
	<i>Cotyledon malacophylla</i>	$n = 12$	„ } 1939.
Begoniaceae	<i>Begonia semperflorens</i>	$n = 33/2,$ $+ 18, + 30, + 33$	* Karyologically polymorphic; Matsuura & Okuno.
	<i>Begonia Rex</i>	$n = 33/2, + 17;$	„ „
Cactaceae	<i>Opuntia polycantha</i>	$n = 11$	Matsuura & Suto, 1935
Umbelliferae	<i>Cnidium ajaense</i>	$n = 12$	„ „ „
	<i>Heracleum lanatum</i>	$n = 12 + f$	„ „ „
	<i>Molopospermum</i>		
	<i>cicutarium</i>	$n = 22$	Wanscher, 1934
	<i>Hydrocotyle vulgaris</i>	$n = 48$	„ „
	<i>Hydrocotyle novae zelandicae</i>	$n = 24$	„ „
Araliaceae	<i>Panax Japonicum</i>	$n = 24$	Wanscher, 1934

Natural Order.	Genus and Species.	Chromosome Number.	Author, Year etc.
GAMOPETALAE			
Caprifoliaceae	<i>Lonicera</i> sp.,	n = 36	Wanscher, 1934
Compositae	<i>Taraxacum</i>		Gustafsson "
	<i>Dahlia Merckii</i>	n = 18	Lawrence, 1931
	<i>D. coronata</i>	n = 16	" "
	<i>D. variabilis</i>	n = 16	" "
	<i>Hidalogoa Wercken</i>	n = 15	" "
	<i>Senecio vulgaris</i>	n = 20	Matsuura & Sato, 1935
	<i>Dahlia coronata</i> ×		
	<i>D. coccinea</i>	n = 16	Darlington, 1937
Campanulaceae	<i>Adenophora</i>		
	<i>remotiflora</i>	n = 18 + 1	" "
Primulaceae	<i>Primula Kewensis</i>		Newton
Ericaceae	<i>Erica cinera</i>	n = 12	
	<i>Phyllodoce coerulla</i>	n = 12	Wancher, 1934
	<i>Ledum groenlandicum</i>	n = 13	" "
	<i>Arctostaphylos</i>		
	<i>uva-wisi</i>	n = 26	Haegerup 1928
Solanaceae	<i>Solanum tuberosum</i>	n = 12	Muntzing, 1933
	<i>Lycopersicum esculentum</i> × <i>L. racemigerum</i>		Afify, 1933
	<i>Nicotiana species</i> *		Lawrence, 1931
Scrophularinaceae	<i>Digitalis purpurea</i>	n = 28	Newton, 1928
	<i>D. ambigua</i>	n = 28	" "
	<i>Verbascum phoeniceum</i>	n = 16	Matsuura & Suto, 1935
	<i>Pedicularis japonica</i>	n = 8	" " "
	<i>Angelonia grandiflora</i>	n = 10	Raghavan and Srinivasan, V. K., 1940

* Secondary pairing between the univalents.

Natural Order.	Genus and Species.	Chromosome Number.	Author, Year etc.
	<i>Calceolaria calibrani</i>	n = 9	Srinath, 1940
	<i>Calceolaria denta</i>	n = 9	" "
	<i>Pentstemon laevigatus</i>	2n = 96	La Cour Cited from Darlington, 1937
	<i>Striga densiflora</i>	n = 20	Kumar and Abraham, 1941
	<i>Striga euphrasioides</i>	n = 20	" " "
Acanthaceae	<i>Asteracantha logifolia</i>	n = 16	Rangaswamy, 1941
Orobanchaceae	<i>Phacellanthus tubiflorus</i>		Matsuura & Toyohuku, 1937

MONOCHLAMYDEAE

Empetraceae	<i>Empetrum herma-</i> <i>phroditum</i>	n = 26	Matsuura & Toyohuku, 1937
	<i>Empetrum nigrum</i>	n = 13	Wanscher, 1934
Salicaceae	<i>Salix basfordiana</i>		Wilkinson
	<i>Populus nigra</i> var. <i>Italica</i>	n = 19	Suto
Euphorbiaceae	<i>Euphorbia granula</i>	4x = 40	Darlington, 1937

MONOCOTYLEDONS

Liliaceae	<i>Veratrum album</i>	n = 16	Haga, 1940
	<i>Veratrum oxysepalum</i>	n = 16	Matsuura & Suto, 1935
	<i>Tricyrtis latifolia</i>	n = 13	Matsuura, 1935
Gramineae	<i>Oryza sativa</i>	4x + 4 = 24	Sakai, 1935
	<i>Oryza sativa</i>		Nandi
	<i>Orza sativa</i>		Ramanujam
	<i>Miscanthus sinensis</i>	n = 19	Takizawa
	<i>M. japonicum</i>	n = 19	"
	<i>M. sacchariflorus</i>	n = 38	"
	<i>M. sieboldi</i>	n = 38	"
	<i>Ehrharta erecta</i>	n = 12	Parthasarathy, 1938
	<i>Ehrharta calycina</i>	n = 24	" "
	<i>Triticum monococcum</i> (haploid variety)		Kihara & Katayama, 1933

Natural Order.	Genus and Species.	Chromosome Number.	Author, Year etc.
<i>Cyperaceae</i>	<i>Carex Pilulifera</i>	n = 9	Heilborn, 1930
	<i>Carex panicea</i>	n = 16	" "
	<i>C. glauca</i>	n = 38	" "
	<i>Carex pilosa</i> } *	n = 32	Okuno, 1940
	<i>Carex pilosa</i> }	n = 57	" "

* According to Okuno, *Carex pilosa* exhibits two different chromosome numbers, n=32 and n=57 and both these exhibit secondary association. In the 32 karyotype associations of two bivalents are more frequent, while in the 57-type associations of 3 bivalents are dominant.

Explanation of Plate I Figures 1-17

1. Early telophase I in *Crescentia Cujete* showing flattened nuclei, $\times 1800$.
2. Interphase nuclei of the same looking spherical and showing 2 nucleoli $\times 1800$.
3. Cytomixis in *Crescentia Cujete* at the tetrad stage, $\times 1800$.
4. Binucleate PMC in *C. Cujete* with crescent-shaped nuclei $\times 1800$.
5. Multinucleate PCM in *C. Cujete* $\times 900$

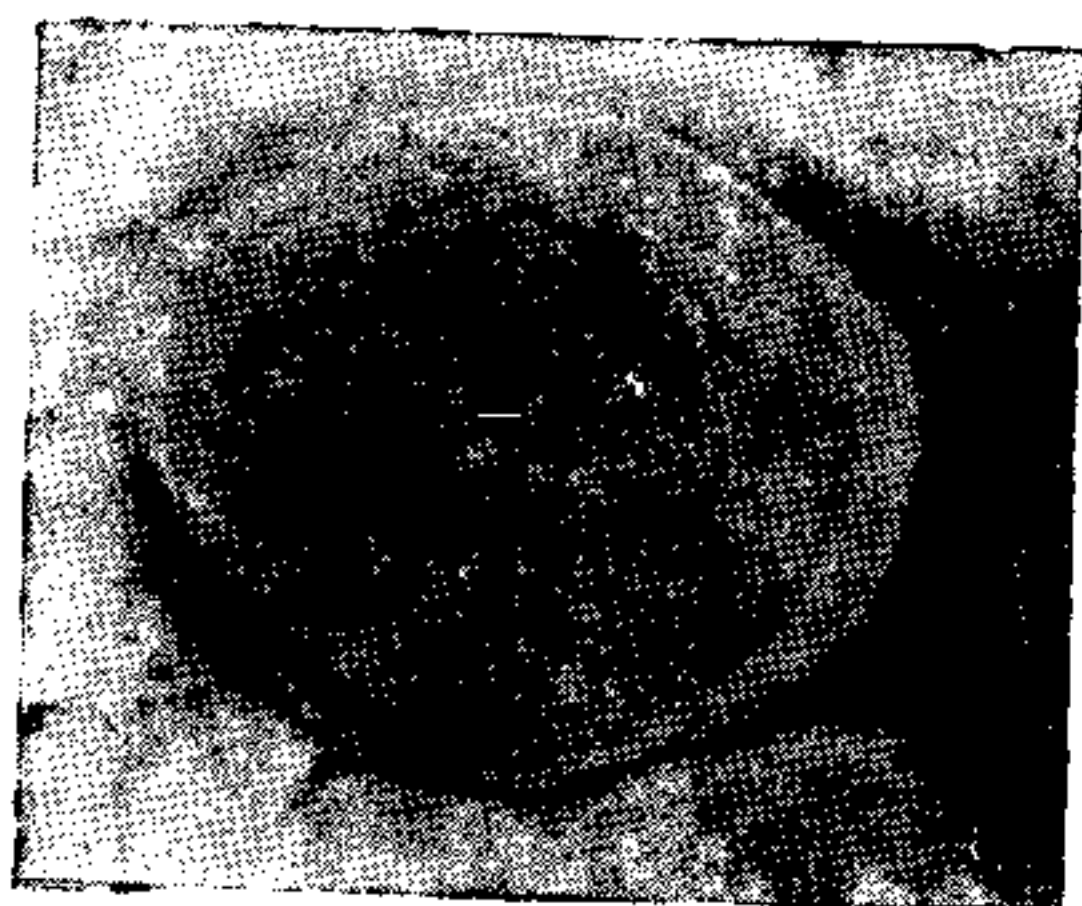
6-11 Secondary association in *Crescentia*
Cujete.

- | | | | |
|-----|--|--|----------------------|
| 6. | Shows an association of | 4(1) 8(2) | in MI. |
| 7. | " " | 5(1) 3(2) 3(3) | MI. |
| 8. | " " | 9(1) 4(2) 1(3) | MI. $\times$ 5200. |
| 9. | " " | 2(1) 6(2) 2(3) | Maximum Association. |
| 10. | " " | Seen in Metaphase II.
9(1) 4(2) 1(3). | |
| 11. | " " | Seen in Metaphase II. | |
| 12. | One of the metaphase plates in <i>Crescentia Cujete</i> showing the maximum association. | | |
| 13. | MI plate of <i>Tabebuia pentaphylla</i> showing secondary Association. | | |
| 14. | MI plate of <i>Tabebuia pentaphylla</i> showing Maximum Association. | | |

15-17. First metaphase plates of *Parmen-
tieria cerifera* showing secondary associ-
ation.

- | | | |
|-----|-------------------------|-----------------------------------|
| 15. | Shows an association of | $7(1) \ 5(2) \ 1(3) \times 2700.$ |
| 16. | „ „ | $1(3) \ 6(2) \ 5(1) \times 2700.$ |
| 17. | „ „ | $2(1) \ 6(2) \ 2(3) \times 2700.$ |
- (Main Association)

PLATE I. 1-5



1



2



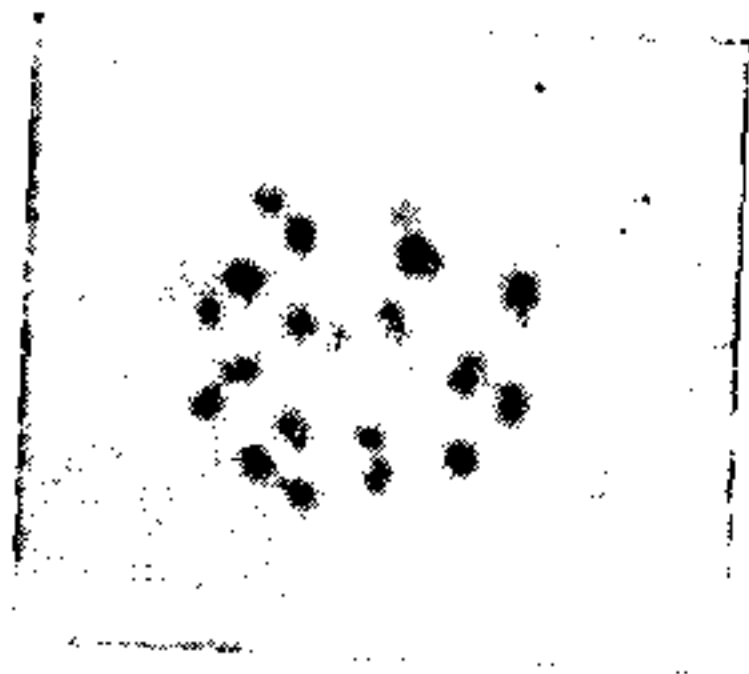
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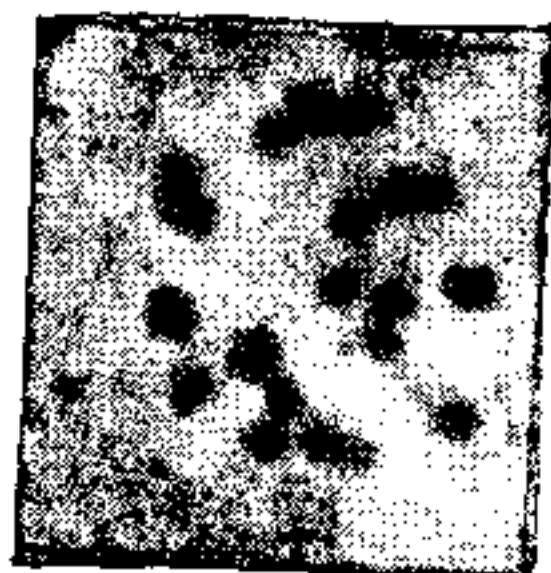
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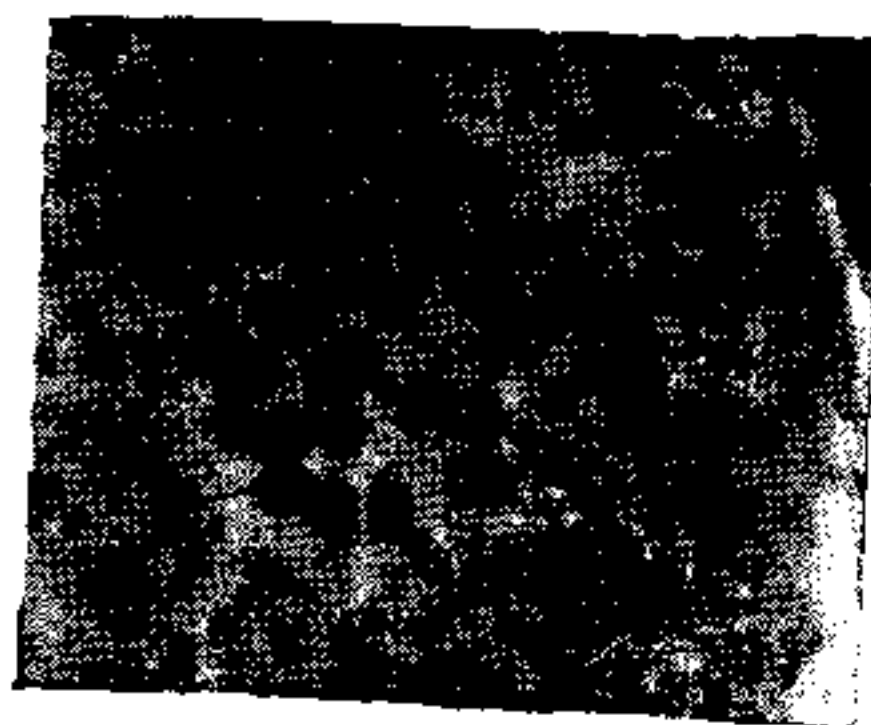
PLATE I. 6-17



6



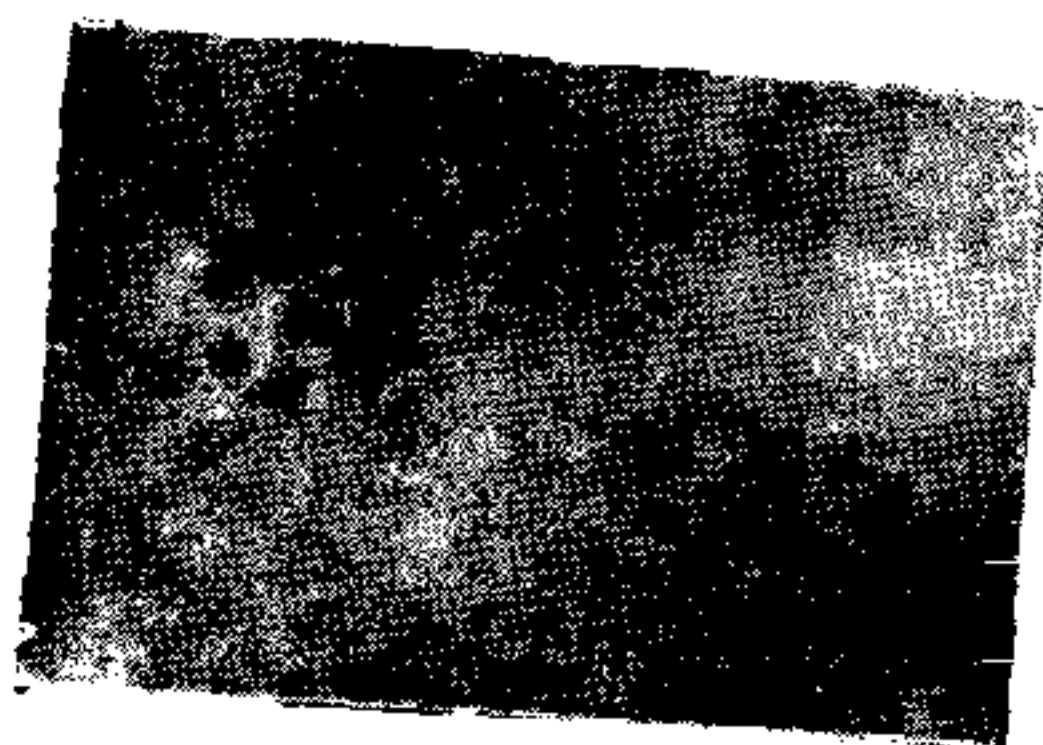
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8



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10



11



12



13



14



PART III

CHROMOSOME NUMBERS AND CYTO-TAXONOMICAL CONSIDERATIONS IN BIGNONIACEAE.*

(with 43 text figures)

* Part III of a thesis accepted for the degree of Doctor of Philosophy of the Annamalai University.

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I. INTRODUCTION.

Chromosome numbers in plants and their correlation to taxonomy seems to have attracted the attention of even the earlier workers. Guignard (1891), has pointed out that the number 16, (this being the diploid chromosome number for three species of the family Orchidaceae which was then available) could form the unit for the entire family Orchidaceae. Guignard's view, that each family represented a unit by itself with a characteristic chromosome number, was more in the nature of speculation; for, chromosome numbers for very few plant species were then available to warrant such generalization.

With increased information, regarding chromosome numbers for the various Angiospermic species, it was soon found that, one number alone cannot characterize a family as was supposed by Guignard. First indications were seen when Strasburger (1882), reported the chromosome numbers 12 and 24 for two different genera belonging to one and the same family—Liliaceae. Subsequently this was found to be true for very many genera, like *Prunus*, *Chrysanthemum*, *Gossypium*, *Triticum*, *Calceolaria* etc., In these genera, the chromosome

numbers were found to exhibit a multiple relationship.

Winge (1917) has made a critical study of the chromosome numbers of a good many of the Angiospermic species. His statistical survey of the haploid chromosome numbers in higher plants led him to the following conclusion. Chromosome numbers in higher plants can be resolved into the prime factors 2 and 3. While such numbers are dominant, the factors 5 and 7 were found to be rather infrequent. The most frequently occurring chromosome numbers in the Angiospermic species, according to Winge, are 8 and 12. Fernandes (1931), has also made a statistical study of the chromosome numbers of especially the higher plants.

Reference to Gaiser's list (1930) and Tischler's list (1931), would reveal the fact that the numbers 7, 9, 11 etc., are also more or less dominant just like the numbers 8 and 12. According to Bruun (1932), "The frequency of the chromosome number of the different species cannot be expressed by any general arithmetical formula; on the contrary, all numbers appear to be within the bounds of possibility".

Winge (1917) has also stated that "Related species have related number of chromosomes". While this may hold good for genera like,

Chrysanthemum, Triticum etc., there are a few cases, where the chromosome numbers exhibit no relationship in speciation. This was found to be the case in the genus *Carex*. Heilborn (1924) has investigated 42 species of this genus and he found 22 distinct types of chromosome numbers. This investigation was further extended to another 40 species by Okuno (1940), where also, it was found that the chromosome numbers fell into 20 different groups ranging from 30 to 90. Thus different species of one and the same genus—*Carex*, (all closely related from taxonomical point of view) bear no relationship at all with reference to their chromosome numbers.

In spite of these variations of chromosome numbers even within a single genus, remarkable instances of parallelism between chromosome numbers and taxonomy (taxonomy based on external morphology) have been met with here and there. The family Scrophularinaceae may be cited to illustrate this point. This includes a number of well defined genera like *Linaria*, *Cymbalaria*, *Chaenorrhinum*, *Antirrhinum* etc. The basic chromosome number in these ranges from 6 to 12 and each one of these genera was found to exhibit a characteristic basic number. Closely allied genera in this family were found to differ from one another in their basic numbers only by one or two

chromosomes. Thus generic distinctions made on the basis of external morphology were found to be supported by chromosome numbers.

In a few cases, correlation between taxonomy and chromosome size has also been established. The genus *Primula*, which has been extensively investigated by Bruun (1932), provides a good example. In the words of the author, "The parallellism between taxonomy and chromosome size, which has been found in almost every part of the genus *Primula*, hardly has its counterpart in any of the genera investigated". There are quite a number of other examples too, to show that chromosome number and chromosome morphology give ample support to taxonomical findings established on external morphology. Langlet (1927, 1932), from a study of more than 200 species of the family Ranunculaceae, arrived at the conclusion, that there is quite a good deal of conformity in the chromosome relations and taxonomical views. Babcock and Cameron (1934) have also found remarkable agreement between taxonomy and chromosome numbers and chromosome morphology in the genus *Crepis*. To quote their words, "Chromosome number and morphology is a taxonomic criterion of great value in this genus". Edgar Anderson (1937), has aptly pointed out that "Taxonomy and cytology study the same phenomenon but from

two different angles" He has also drawn an analogy between the two (Karyology and Taxonomy) namely, that taxonomy is something like getting an insight into a family of strangers from a casual meeting of the members of the family in the street; while, karyology is comparable to looking and studying the members of the family of strangers in their cellar windows." From all these, it would appear that chromosome number, morphology etc., possess deeper significance in taxonomy than has been thought of hitherto.

The present work is an attempt to find out, if chromosome numbers in this little-investigated family would throw any light as to relationships of the various genera established on morphological grounds by Systematists and also the manner in which the various species could have arisen.

Cyto-taxonomical investigations, have their premise, in the constancy of chromosome number for a given species. No doubt, there are several plant species which have revealed the presence of what may be called oscillating numbers. Randolph (1928) found that some strains of *Zea Mays* exhibited chromosome numbers ranging from 20 to 28. Clausen (1931) in *Viola canina* has reported variable number

of fragments besides the 40 somatic chromosomes. According to Cameron (1934), *Crepis syriaca* shows varying number of chromosomes ranging from 10 to 18. Tischler (1937), has referred to the unpublished results of Wulff, namely, that different ecotypes would exhibit different numbers. Thus it has been found by Wulff that *Jasione montana* growing in sand-dunes, regularly exhibited 7 haploid chromosomes while the same species found elsewhere in a different habitat, showed only 6 haploid chromosomes. Three karyotypes of *Scilla indica* exhibiting $2n=44$, 45 and 46 have been reported by Raghavan and Venkatasubban (1939). Okuno (1940), has recorded two chromosome numbers, $n=32$ and $n=57$ for the same species, *Carex pilosa*. Such cases have to be treated as mere exceptions in cyto-taxonomical considerations.

In cyto-taxonomical problems, in addition to chromosome number, chromosome morphology, number of chiasmata formed during diakinesis, genetical data, data from plant geography and oecology etc., have to be taken into consideration to make the study more complete. Of these, chromosome morphology has to be singled out, since it has proved to be a sound basis for determining the phylogeny of species. The phylogeny of the genus *Crepis* has been attempted on this basis with remarkable success

by Babcock and Cameron (1934). But it should be remembered, that only very few plants are in possession of small number of chromosomes which are also at the same time equally big as in *Crepis*. Therefore, in most cases, attempts to classify the various genera and species have to be made only with reference to mere chromosome numbers. This is especially so, in those cases where the chromosomes are not only found in large numbers but also comparatively small, as in the Bignoniaceous species.

No doubt, one cannot draw any important or valid conclusions regarding relationships among the genera and species, merely on the basis of the chromosome numbers unsupported by evidence from genetical studies, geographical distribution of the species etc. The investigations of Heitz (1926) on the comparative size, and number and form of chromosomes, have brought home this point, namely, that chromosome number alone is an inadequate criterion in the determination of relationships. The same idea is also emphasized by Babcock (1934). To quote his own words, "A mere study of the distribution of the chromosome numbers in a small fraction of the species in a family or genus and especially without due consideration of other evidence on taxonomic relationships in those groups, may lead to quite erroneous conclusions concerning the basic number."

If chromosome number is no guide to affinity, the same may be said of chromosome morphology also. According to Heitz (1926), related species of the genus *Cyclamen* were found to show dissimilarities in the size of their chromosomes. Fagerlind (1937) has also reported the same phenomenon. According to him, the descendants of a cross between *Galium Mollugo* types with different chromosome numbers and different sized chromosomes, did not exhibit any of the chromosome differences of the parents. On the other hand, the size differences of the chromosomes of the parents were found to be levelled out in the hybrids. Fagerlind regarded that the 'matris material' was distributed proportionally over the chromosomes present in the hybrids. In such cases, neither the chromosome number nor chromosome morphology would be of use as guide to affinity.

This can readily be understood, if it is borne in mind, that the chromosomes even in a species are likely to undergo evolution. Thus on the hypothesis of Lewitsky (1931) and of Levan (1933), primitive species would exhibit only chromosomes having median or sub-median constrictions, while the advanced ones may show terminal or sub-terminal constrictions, even in allied species. Therefore, two closely related species may show differences in their

chromosome morphology with or without numerical differences in their chromosome numbers.

From what has been said above, it would appear, that the different aspects of the chromosomes like, number, size etc., when studied separately, is not likely to throw much light on plant relationships. The various features should be studied with reference to comparative morphology, geographical distribution, cytogenetics, etc., and unless results from these different sources of study are correlated, no valid conclusions regarding phylogeny could be drawn.

It should be mentioned in this connection, that important conclusions have however, been reached through a study of chromosome numbers as in the genus *Viola* by Clausen (1927), *Kalanchoe* by Baldwin (1938) and Beard (1937) in *Cactaceae*. Tischler (1928, 1929) himself while emphasizing the supreme importance of chromosome morphology and chromosome numbers as guiding factors in systematic botany, has introduced the well known classification, the *Pinus-type*, the *Chrysanthemum-type*, the *Carex-type* and the *Antirrhinum-type*, all based on chromosome numbers only.

Chromosome numbers have been determined for very few species in this family so

far. Reference to Tischler's lists (1935, '36, 1938), Merton Catalogue (Maude 1939), Gaiser's list (1926 and 1930), List of chromosome-numbers of plants cultivated in Japan (Kihara, Yamamoto and Hosono, 1931), Idiogram study in Phanerogams (Matsuura and Suto 1935) and Studies on the chromosome numbers in Higher plants with special reference to Cytokinesis by Sugiura (1936), were made and it was found that information on chromosome numbers in this family is limited to only the following:—

<i>Campsis radicans</i>	n = 20
<i>C. grandiflora</i>	n = 18
<i>Incarvillea grandiflora</i>	n = 9
<i>I. Delavayi</i>	n = 9
<i>Tecoma capensis</i>	n = 17
<i>T. jasminoides</i>	n = 19
<i>T. Smithii</i>	n = 18
<i>T. Tagliabuana</i>	n = 20
<i>Catalpa syringifolia</i>	n = 20
<i>Bignonia venusta</i>	Ca n = 25

To this meagre list are added through the present investigation, chromosome numbers for another 35 species spread over 16 genera. Out of the 16 genera referred to, 6 come under the tribe Bignonieae, 7 under Tecomeae and 4 under Crescentieae. The tribes, Eccremocarpeae which consists of a single genus *Eccremocarpus*, and Tourrettieae which is also

monogeneric, had to be omitted owing to want of material.

II. Materials and Methods :—Most of the plant materials for the present investigation were obtained from the Botanical Garden Sibpur, Calcutta, and from the Botanical Garden, Ootacamund. A few were obtained from the United States Department of Agriculture, Bureau of Plant Industry, Washington, through the courtesy of Professor R. R. Gates, F.R.S., to whom my grateful thanks are due. Owing to the availability of only seedlings of most of the species for the present study, chromosome numbers could be determined only from the cells of the root tips; but wherever possible, these numbers were checked by meiotic counts. Root tips not exceeding 2-3 mms., in length were washed in distilled water, to remove all adhering soil particles and after a prefixation in Carnoy's fluid for about 30 seconds, were fixed in Navashin's fluid for a period of 24 hours. After this interval, they were thoroughly washed in water and gradually dehydrated using different grades of ethyl alcohol. For the infiltration of paraffin wax, chloroform was used as the solvent. Sections were cut at a thickness of 14-16 microns and stained exclusively in Newton's Iodine Genetian Violet. To get cells with good metaphase plates for counting, was a matter of

considerable difficulty and the material had to be fixed at different intervals of time.

III. **Observations : (a) Pro-chromosomes.**

Pro-chromosomes have been observed in the meristematic cells of the root tips, of almost all the species examined, in the present studies. The tapetal cells of a few species like *Kigelia pinnata* and *Crescentia Cujete* also exhibited the presence of these. Detailed observations of these bodies were made while studying the somatic mitosis in *Oroxylum indicum*. This species has a diploid chromosome number of 30. Most of them show either sub-median or sub-terminal constrictions.

Some amount of literature has accumulated on the nature and functions of prochromosomes, since Rosenberg (1904) for the first time drew attention to these bodies in *Capsella* and *Cucurbita*. The papers by Heitz (1929), Grégoire (1932), Doutreligne (1933), Manton (1935) and Raghavan (1938) are some of the recent important contributions on the subject.

The prochromosomes or the 'Euchromosomes' of Grégoire (1932) are generally referred to as the 'organic representatives' of the chromosomes. They are believed to represent definite portions of the chromosomes namely,

the region of the attachment constriction. According to Raghavan (1938), the prochromosomes are directly derived from the telophase chromosomes. The persistence of these bodies throughout mitosis and their numerical correspondence with the chromosomes, are strongly in favour of the view, that these represent the region of the centromeres.

Though no critical study could be made with the present material, it may be mentioned that some of the stages obtained, bear a close resemblance to what have been figured and described for *Polanisia trachysperma* (Raghavan 1938) and *Alpinia calcarata* (Raghavan and Venkatasubban, 1941).

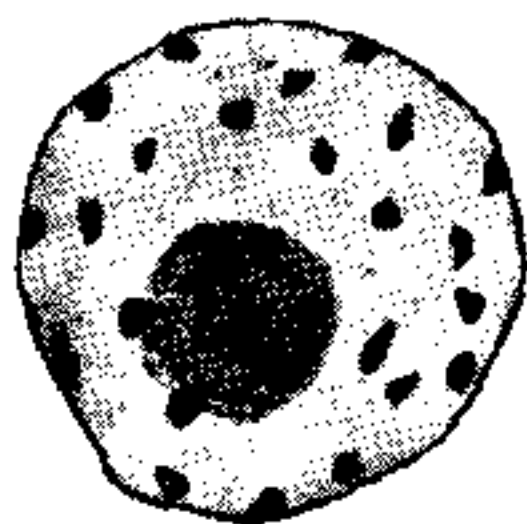
In the resting condition (text fig. 35), there were seen about 27 bodies. These were only faintly stained. The determination of the exact number of these, was a matter of considerable difficulty, owing to their peripheral distribution. Therefore, the number given here is only approximate. There was found only one nucleolus and this was spherical and clearly visible. Four prochromosomes were seen attached to the nucleolus. With the onset of prophase, there appeared to be a slight increase in the nuclear volume and the bodies which were rather faint became conspicuous now, presumably owing to their increased chromaticity. In

a few preparations a faint reticulum connecting these bodies was noticed' (text fig. 36). As prophase advanced, the rod-shaped bodies became irregular in their outline. They showed attenuated ends, which would suggest that these bodies have undergone a stretching process (text figs. 37 and 38). The nucleolus which was till now spherical appeared to have suffered a change of shape and became irregular (text fig. 38). It has also been observed at this stage that more of the prochromosomes were drawn towards the nucleolus than before. At late prophase these became more elongated (text fig. 39) and at a still later stage, the constrictions too, became visible (fig. 40). In the prometaphase, the nuclear membrane disappeared and the chromosomes presented a clumped appearance, often ascribed to bad fixation. During the prometaphase and metaphase stages, the chromosomes seem to undergo a certain degree of contraction. In metaphase, 30 rod-shaped chromosomes with submedian or subterminal constrictions were visible (text fig. 41). The chromosomes spread out at this stage and did not touch one another. In telophase (text fig. 42), the chromosomes once again became very small and less chromatic and assumed a peripheral position. Prochromosomes were quite visible now. There was not much difference between this stage and the stage

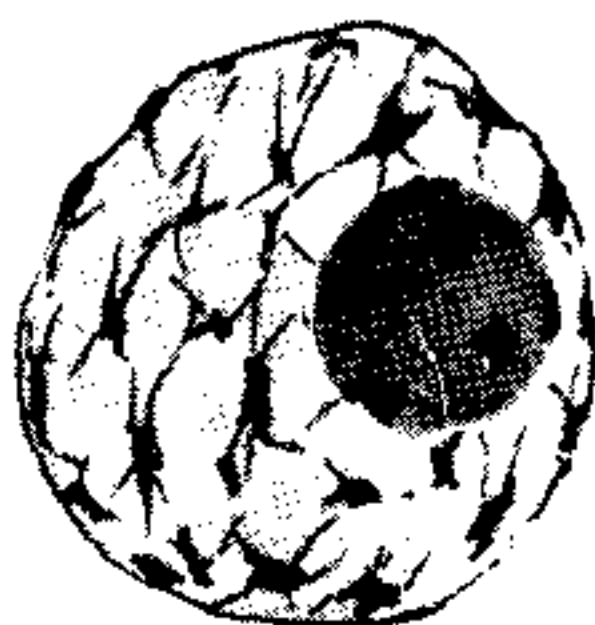
Text figures 35-43

Stages in the somatic mitosis of *Oroxylum indicum*, showing prochromosomes.

35. Resting condition; the attachment of 4 prochromosomes to the nucleolus and about 27 prochromosomes are seen.
36. Early prophase. The peripheral distribution of the prochromosomes and the fibres are seen.
37. Prophase showing the attenuated ends of the prochromosomes.
38. Mid-prophase, showing the irregular outline of the nucleolus and the accumulation of more prochromosomes around the nucleolus.
39. Late prophase showing the elongated prochromosomes.
40. Early prometaphase where the chromosomes are seen somewhat contracted; the constrictions are visible now.
41. Metaphase showing 30 chromosomes.
42. Early telophase, showing the reappearance and the peripheral distribution of the prochromosomes. In one of these 4 nucleoli of different sizes are seen.
43. Late telophase showing two nucleoli, one big and the other small. The peripheral distribution of the prochromosomes is also evident.



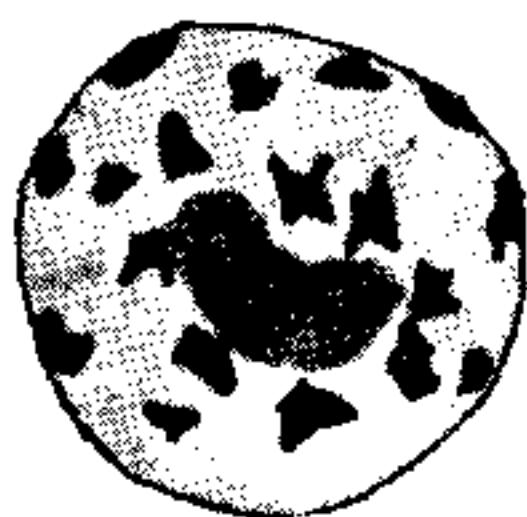
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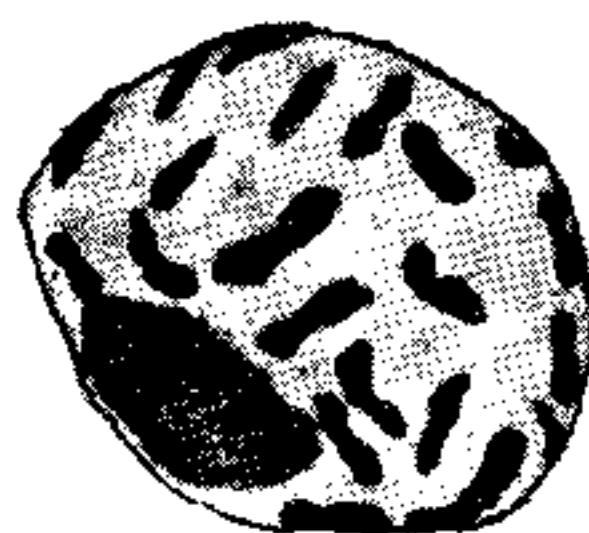
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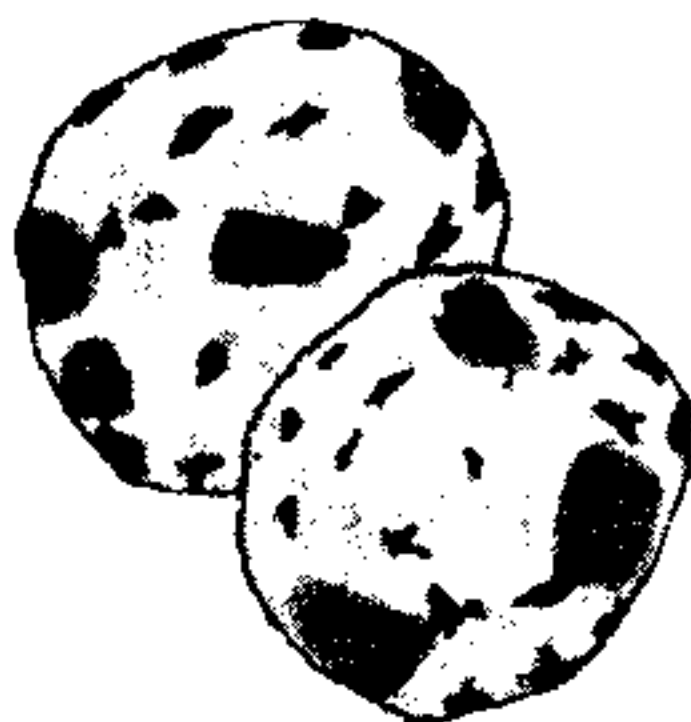
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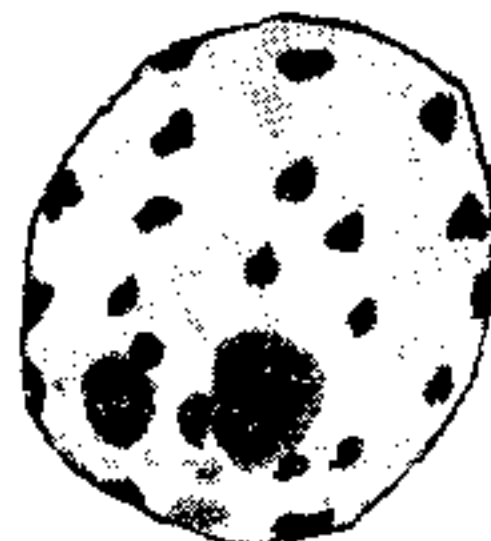
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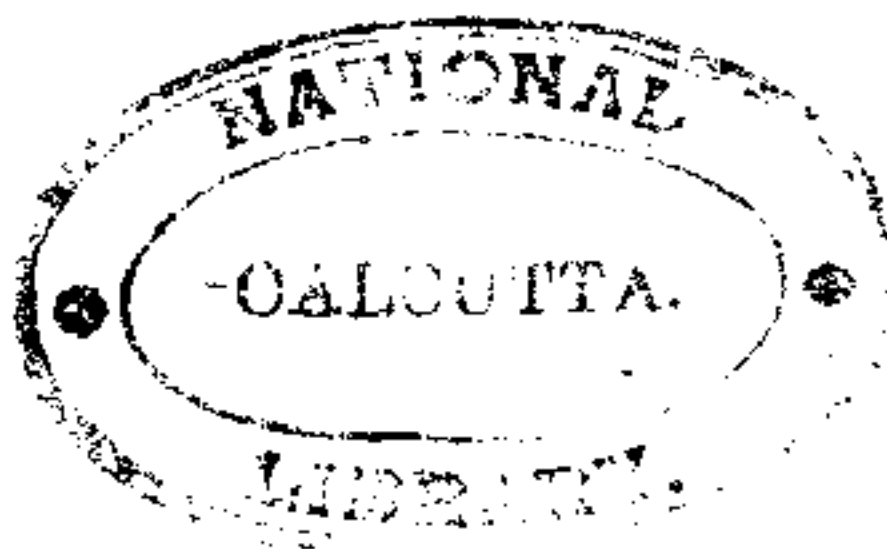


42



43

2, 10



with the nucleous at the resting condition, except that 4 nucleoli were seen instead of one. Text fig. 43, which represents late telophase, shows the presence of only 2 nucleoli instead of 4. This may be due to fusion having taken place among the nucleoli. These two nucleoli might have fused in their turn to give rise to the solitary nucleolus, seen at the resting stage.

From the foregoing description, there is no room to doubt, that the prochromosomes represent, that portion of the chromosome, adjacent to the centromere. Depending upon the position of the centromere, the prochromosomes present either a constricted or non-constricted appearance. The attachment constriction presumably remains chromatic throughout the mitotic cycle and is therefore recognizable, as the so called prochromosomes, even during the resting stage of the nucleus.

The question as to what happens to the rest of the chromosome during the mitotic cycle has been described by Raghavan (1938) at some length. According to him, the nucleolus is organized by satellited chromosomes as usual; but after the organization of the primary nucleolus, a portion of the chromatin contained in the chromosomes, was found to accumulate on the surface of this primary nucleolus, in the form of darkly stained peripheral patches, from late telophase to early prophase. The close

association of the prochromosomes with the nucleolus at telophase and early prophase was explained on this basis. This means to say that, while the chromatic material persists throughout the mitotic cycle only at the region of the centromere, the rest of the chromosome gives up its chromaticity and this is stored upon the periphery of the primary nucleolus. In the present investigation, such a crowding of the bodies was noticed, though no distinct nucleolar patches such as were described by Raghavan (1938), could be recognized. This crowding would appear to support the above mentioned interpretation.

The presence of the fibres, which are obviously the chromonemata, the numerical correspondence of the prochromosomes to that of the chromosomes, the organization of the primary nucleolus and the crowding of the prochromosomes around it to give up their chromaticity, all these would indicate that chromosome structure as well as chromosome cycle, in plants with prochromosomes, are in essence, the same as in plants without the prochromosomes.

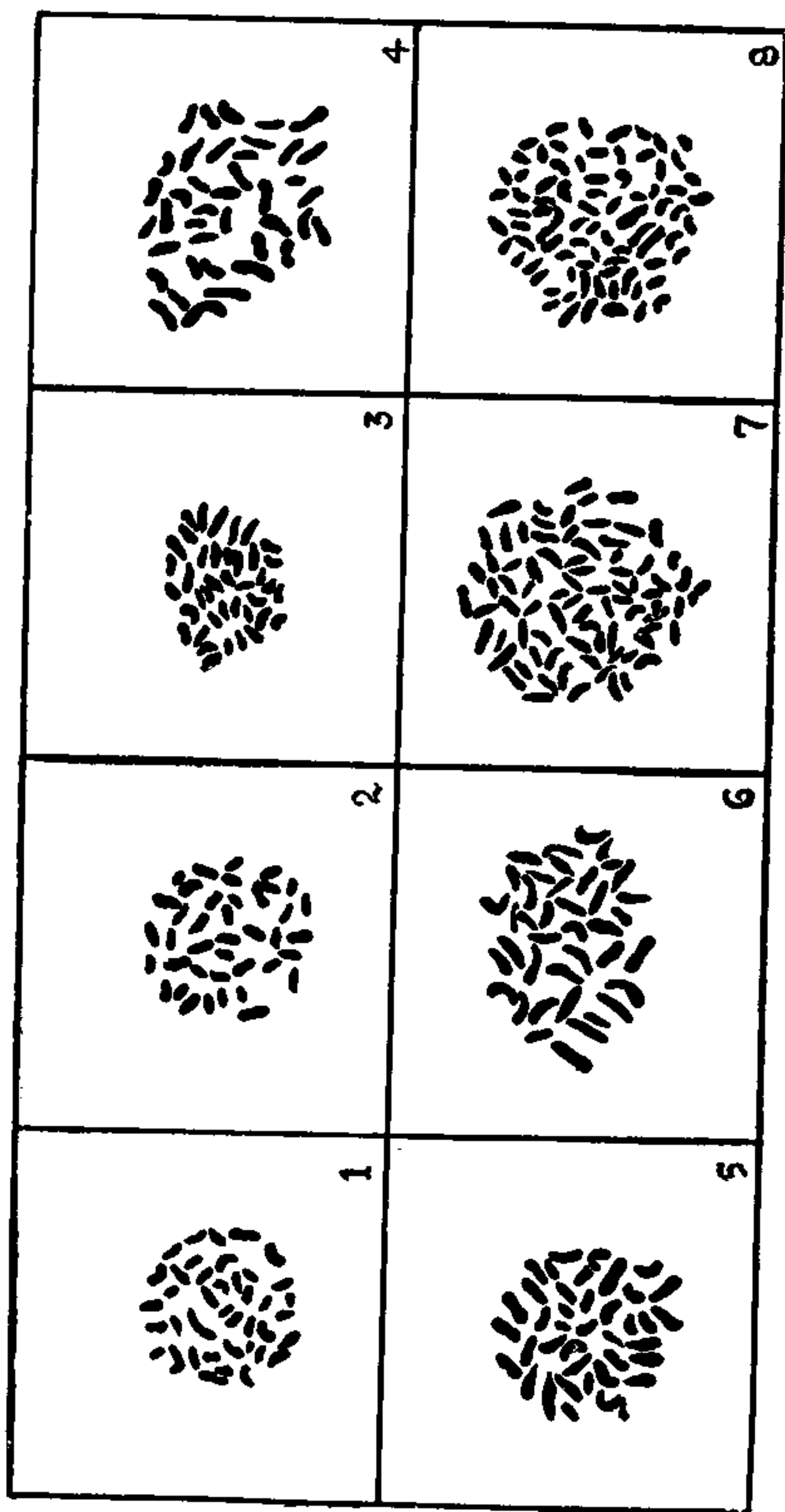
(b) Chromosome Numbers and Morphology :— Text figures 1-34, represent the drawings of somatic metaphase plates of the different species studied. It may be seen from these figures,

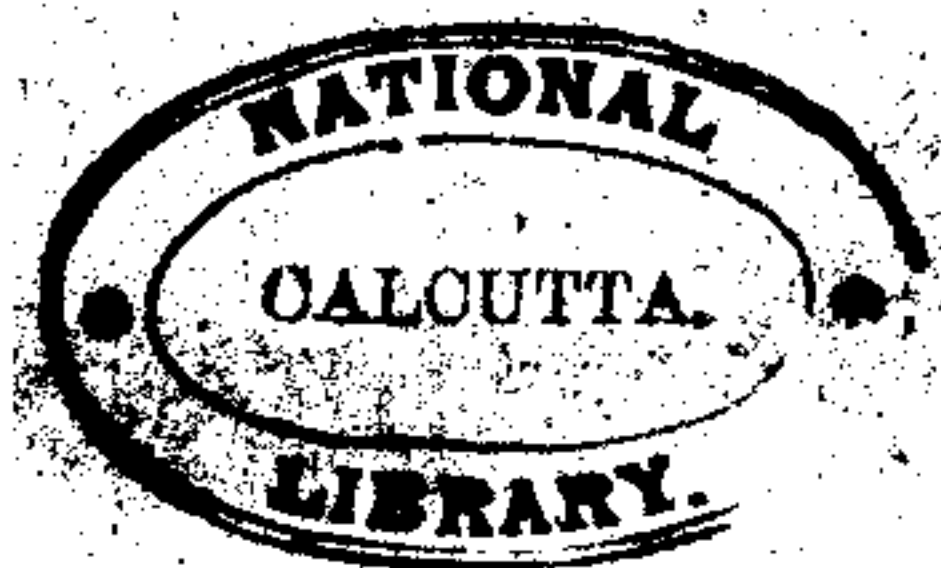
Legend to text figures 1-43.

(All figures have been drawn with a camera lucida at an approximate magnification of $\times 3600$. The original drawings (figures 1-34) have been reduced to about $\frac{3}{4}$ while figs., 35-43 have been enlarged to $1\frac{1}{3}$ in the photographs.)

Text figures 1-8:— Somatic metaphase plates.

1. *Bignonia magnifica*, $2n = 40$.
2. *B. megapota mica*, "
3. *B. diversifolia*, "
4. *B. Chamberlaynii*, "
5. *B. Cherere*, "
6. *B. purpurea*, "
7. *B. gracilis*, $2n = 80$
8. *B. Tweediana*, "





that the chromosomes present more or less a uniform appearance. The chromosome number also is more or less the same; the majority of them exhibit a diploid chromosome number of $2n=40$. Though a large number of plates were examined for each of these species, no variation in the chromosome numbers was observed. The somatic division appears to be very regular in most of these cases. Satellites too were not visible in any of the preparations except in *Tecoma capensis* (text fig. 23), where one of the chromosomes showed a trabant.

Table 1, gives a list of chromosome numbers for this family known so far. In the table an asterisk mark is placed against those species, for which chromosome numbers have been determined by previous workers. From the list it may be seen, that out of the 44 species for which chromosome number is now recorded, as many as 27 show a diploid number of $2n=40$. This number, $2n=40$, therefore occupies a dominant position in this family. There are only four species which exhibit a diploid chromosome number above 40. The rest of the species show varying number of chromosomes, but less than 40, in their root tip cells. The lowest diploid number $2n=18$, has been found in two species of *Incarvillea*,—*I. grandiflora* and *I. Delavayi*.

TABLE No. I

Genus and species.	Chromosome numbers		Reference.
	n	2n	
TRIBE BIGNONIEAE			
Genus <i>Bignonia</i>			
<i>Bignonia Cherère</i> , Lindl.	—	40	
<i>B. magnifica</i> , Bull.	20	40	
<i>B. Chamberlaynii</i> , Sims.	—	40	
<i>B. megapotamica</i> , Spreng.	20	40	
<i>B. diversifolia</i>	—	40	
<i>B. purpurea</i> , Hook.	—	40	
<i>B. venusta</i> , Ker. *	25	—	Duggar, B.M., 1899
<i>B. gracilis</i>	—	80	
<i>B. Tweediana</i> , Lindl.	—	80	
Genus <i>Adenocalymma</i>			
<i>Adenocalymma calycina</i>	—	40	
Genus <i>Amphilophium</i>			
<i>Amphilophium Mustisii</i> , H.B.S.K.	—	44	
Genus <i>Tanaecium</i>			
<i>Tanaecium albiflorum</i> , DC	—	40	
Genus <i>Oroxylum</i>			
<i>Oroxylum indicum</i> , Vent.	—	30	
Genus <i>Millingtonia</i>			
<i>Millingtonia hortensis</i> , Linn.	15	30	
TRIBE TECOMEAE			
Genus <i>Tecoma</i>			
<i>Tecoma stans</i> , Juss.	20	40	
<i>T. radicans</i> , Juss.	—	40	
<i>T. Tagliabuana</i> *	20	—	de Vilmo- rin and Simonet 1927

<i>T. chrysántha</i> , DC.	—	38	
<i>T. grandiflòra</i> , Del.	—	38	
<i>T. ròsea</i> , Bertol. Fl. Guatim	—	38	
<i>T. austràlis</i> , R. Br.	—	38	
<i>T. Smithii</i> , W. Wats *	18	36	Sugiura, 1936
<i>T. capénsis</i> , Lindl.	17	34	
Genus Dolichandrone			
<i>Dolichandrone Rheedii</i> , Seem.	—	40	
<i>D. stipulata</i> , Benth.	—	40	
<i>D. platycalyx</i> , Baker	—	40	
Genus Tabebuia			
<i>Tabebuia guayacan</i> , Hemsl.	—	40	
<i>T. rosea</i> , DC.	—	40	
<i>T. pentaphylla</i> , Hemsl.	20	—	
<i>Tabebuia species</i> (Sibpur)	—	40	
Genus Campsis			
<i>Campsis radicans</i> , Seem *	—	40	
<i>C. grandiflora</i> *	—	36	
Genus Incarvillea			
<i>Incarvillea grandiflora</i> *			
Bur and Franch	—	18	Sugiura, 1936
<i>I. Delavàyi</i> , * Bur and Franch	—	18	Sugiura, 1936
Genus Jacaranda			
<i>Jacaranda mimosaeifolia</i> , D. Don.	—	36	
Genus Spathodea			
<i>Spathodea campanulata</i> , Beauv.	13	—	Raghavan and Venkatasubban, 1940
Genus Catalpa			
<i>Catalpa syringifolia</i> , Sims. *	20	—	
Genus Stereospermum			
<i>Stereospermum chelonoides</i> DC.	20	40	

Genus *Pajanelia*

<i>Pajanelia Rheedii</i> , DC.	—	40
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TRIBE CRESCENTIEAE

Genus *Parmentiera*

<i>Parmentiera edulis</i>	—	40
<i>P. cerifera</i> , Seem.	20	40

Genus *Crescentia*

<i>Crescentia Cujete</i> , Linn.	20	40
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Genus *Kigelia*

<i>Kigelia pinnata</i> , DC.	20	40
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Genus *Phyllarthron*

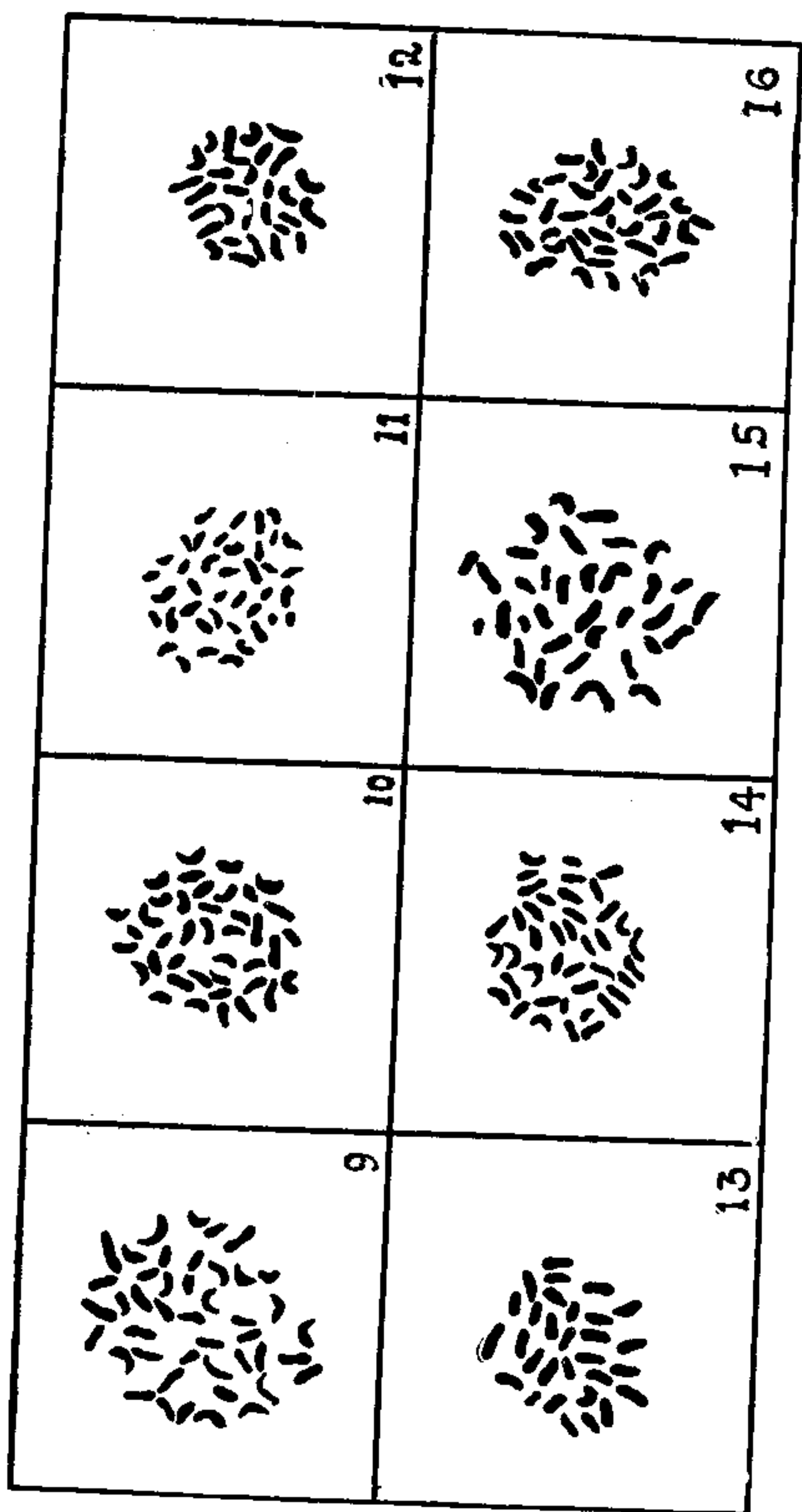
<i>Phyllarthron comorense</i> , DC.	—	40
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Reference to table I will show, that the number of chromosomes found in the tribe *Bignoniaceae* are, 30, 40, 44, 50 and 80. Of these, the number 40 was the most frequent one. Out of the nine species of *Bignonia* examined, 6 showed a diploid chromosome number of 40, and two species (*Bignonia gracilis* and *B. Tweediana*), revealed the typical polyploid number 80. The haploid number, $n=25$ reported for *Bignonia venusta* by Duggar (1899), has to be regarded only as an approximate count, since the counting was made during the first anaphase. Whether the number 25 is correct or not, could not be verified for want of material.

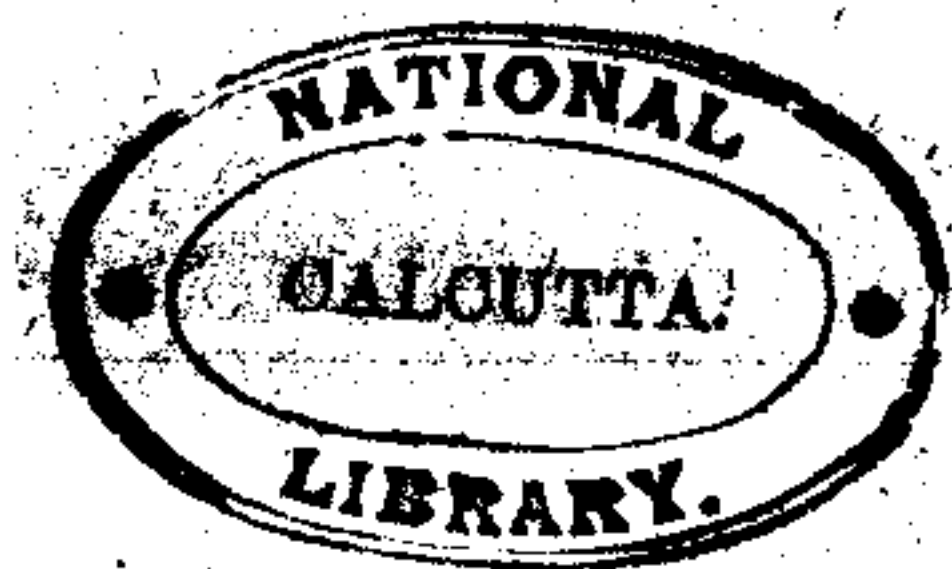
Amphilophium Mustisii was found to possess 44 rod-shaped chromosomes in the root tip cells (text fig. 9). The chromosomes were

Text Figures 9-16 :—Somatic Metaphase Plates.

9. *Amphilophium Mustisii*, $2n = 44$
10. *Adenocalymna calycina*, $2n = 40$.
11. *Pajanelia Rheedi*, $2n = 40$.
12. *Oroxylum indicum*, $2n = 30$.
13. *Millingtonia hortensis*, $2n = 30$.
14. *Tanaecium albiflorum*, $2n = 40$.
15. *Jacaranda mimosaeifolia*, $2n = 36$.
16. *Stereospermum chelonoides*, $2n = 40$.



3 μ



comparatively big and bore a close resemblance to those observed in *Bignonia purpurea* and *Bignonia Chamberlaynii* (text figs. 6 and 4). In both *Oroxylum indicum* and *Millingtonia hortensis*, a diploid chromosome number of $2n = 30$ was observed. The somatic complements also exhibited a close resemblance in these two species (Compare text figures 12 and 13).

The different members of the tribe *Tecomeae* also exhibited a diploid chromosome number of $2n = 40$. In this respect this tribe closely resembles *Bignoniaceae*. Out of the 25 species of the tribe *Tecomeae* investigated, as many as fourteen species were found to reveal the diploid chromosome number 40. The 40-chromosomed members are, the three species of *Dolichandrone*, the four species of *Tabebuia*, three species of *Tecoma*, *Campsis radicans*, *Catalpa syringifolia*, *Stereospermum chelonoides* and *Pajanelia Rheedii*.

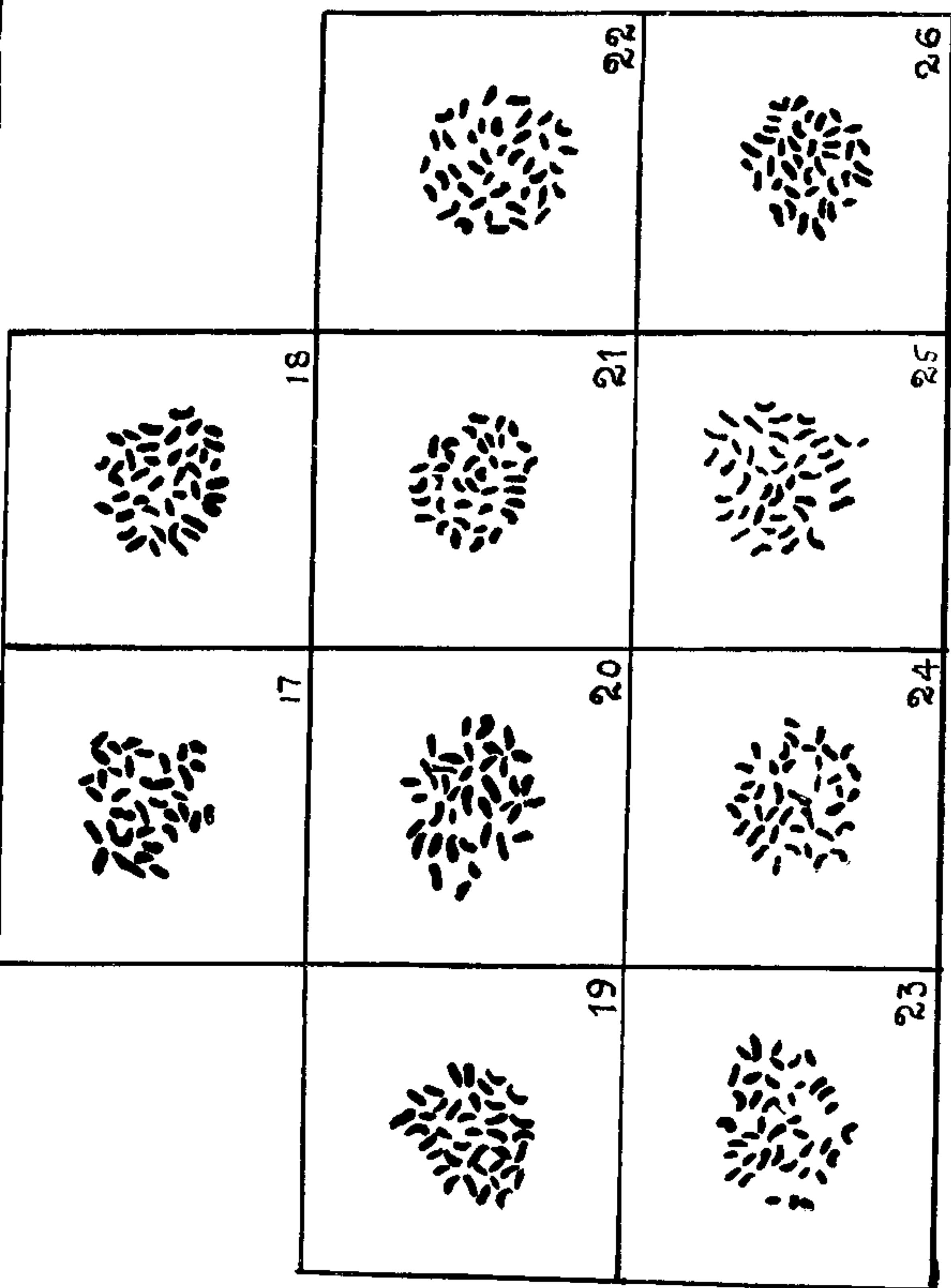
Lesser chromosome numbers than $2n = 40$ were also met with among the members of the tribe *Tecomeae*. For example, the somatic chromosome number, $2n = 38$ was found to characterize, *Tecoma chrysantha*, *Tecoma grandiflora*, *T. rosea* and *Tecoma australis*. The number $2n = 36$ was observed in *Tecoma Smithii* and *Jacaranda mimosaeifolia*. *Tecoma capensis* revealed a diploid chromosome num.

ber of $2n=34$ and this is the lowest number recorded so far as the genus *Tecoma* is concerned. In *Spathodea campanulata*, 13 haploid chromosomes were counted both in the first and second metaphase by Raghavan and Venkatasubban (1940 a). The lowest number recorded with reference to the different members of this family, is $2n=18$. This was found in *Incarvillea grandiflora* and *I. Delavayi* (Sugiura, 1936).

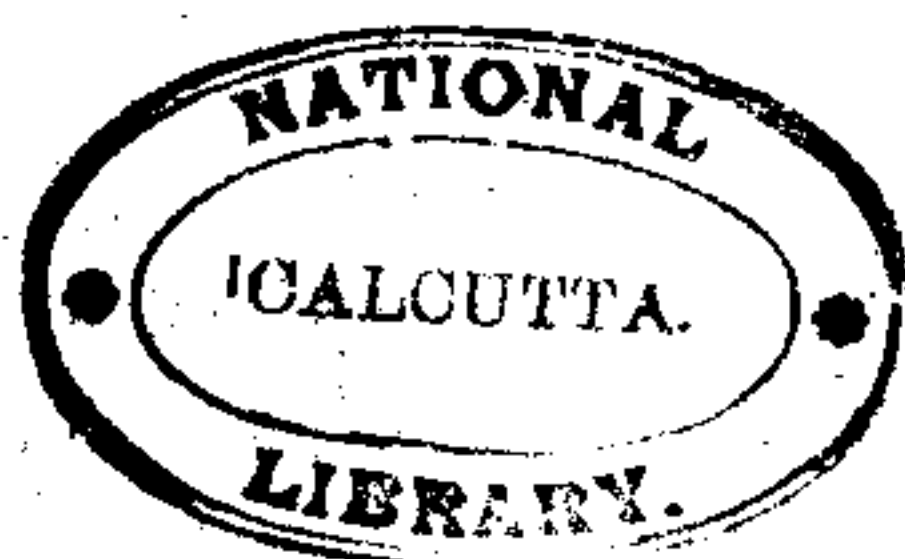
Only four genera of the tribe CRESCENTIEAE were available for examination. In *Crescentia Cujete*, *Parmentiera cerifera* and *Kigelia pinnata*, both diploid and haploid numbers were determined. The diploid chromosome number for these was found to be $2n=40$ while the haploid number 20 was also found. The same diploid number, $2n=40$ was also observed in *Phyllarthron comorense*. Thus there appears to be a greater uniformity among the members of the tribe CRESCENTIEAE, from the point of view of mere chromosome numbers, than the rest of the tribes. The somatic chromosomes in *Parmentiera edulis* (text fig. 30), *Parmentiera cerifera* (text fig. 31), *Phyllarthron comorense* (text fig. 32), *Crescentia Cujete* (text fig. 33) and *Kigelia pinnata* (text fig. 34), also revealed a striking resemblance in their morphology.

Text Figures 17-26

17.	<i>Tecoma capensis</i> ,	$2n = 34.$
18.	<i>Tecoma grandiflora</i> ,	$2n = 38.$
19.	<i>T. rosea</i> ,	"
20.	<i>T. australis</i> ,	"
21.	<i>T. chrysantha</i> ,	"
22.	<i>Tecoma radicans</i> ,	$2n = 40.$
23.	<i>Tecoma stans</i> ,	"
24.	<i>Tabebuia rosea</i> ,	"
25.	<i>T. guyacan</i> ,	"
26.	<i>Tabebuia</i> (species ?)	"



3 μ



Since chromosome analysis, with reference to the position of the centromere, could not be attempted owing to the large number of chromosomes and their extremely small size, the total length of the chromosomes in some of these species was measured and on this basis, they were analysed roughly into long medium and short ones. Table II shows the result of the analysis.

From the table, it will be seen that, the total length of the entire somatic complement, in the different species, ranges from 108 mms., to 115 mms. Only in two instances this range was exceeded. *Tabebuia guayacan* had a total length of 125·2 mm. while for *Amphilophium Mustisii*, it was found to be 162·1 mm. Though the total length of the somatic chromosomes in the different species was found to be approximately the same, they were not identical, as can be seen from the different frequencies of the long, medium and short chromosomes. For instance, in *Oroxylum indicum*, there were found 16 long-chromosomes of length 4·5 mm., 12 medium-sized chromosomes of length 2·8–3·4 mm. and two very short-chromosomes of about 2·4mm. in length. All these would give a total length of 108 mm. In *Millingtonia hortensis*, which has the same chromosome number as *Oroxylum indicum*, there were found only 10 long-chromosomes of length 3·9–5 mm., 18

medium-sized chromosomes of length 3.2–3.7 mm. and two small-chromosomes of about 2.5 mm. in length. The total length of the entire complement in this case was found to be about 106.1 mm.

The analysis presented in table II has been found to be of some value; especially the data, with reference to the various *Tecoma* species, which exhibit a regular aneuploid chromosome series, like 34, 36, 38 and 40. From the data gained through the measurement of the relative lengths of the chromosomes in these, some light has been thrown, as to the possible manner by which such an aneuploid series have arisen. These are discussed further down in the body of this paper.

Text Figures 27-34

27.	<i>Dolichandrone platycalyx</i> ,	2n = 40..
28.	<i>D. stipulata</i> ,	"
29.	<i>D. Rheedii</i> ,	"
30.	<i>Parmentiera edulis</i> ,	"
31.	<i>P. cerifera</i> ,	"
32.	<i>Phyllarthron comorense</i> ,	"
33.	<i>Crescentia Cujete</i> ,	"
34.	<i>Kigelia pinnata</i> ,	"

 27	 28	 29	 30
 31	 32	 33	 34

3μ

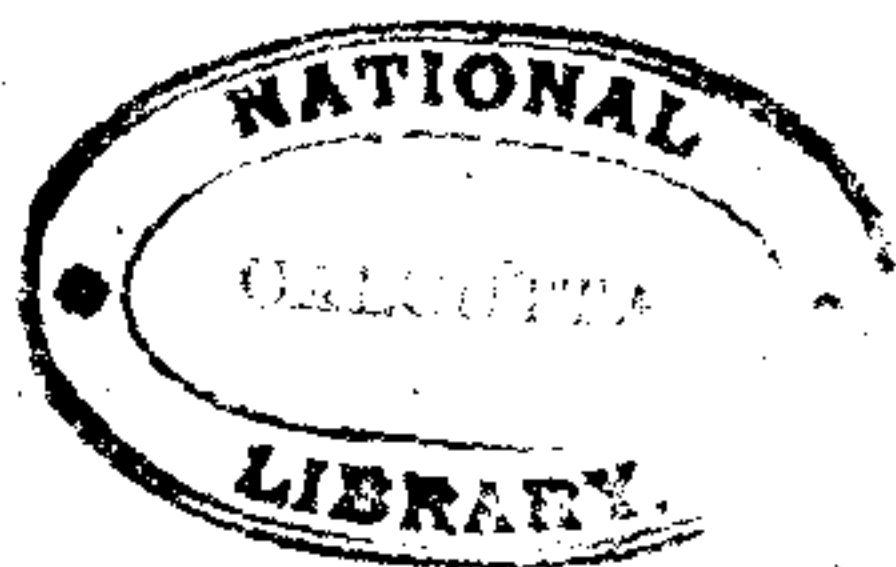


TABLE II.

Showing chromosome analysis

Genus and species.	No. of long chromosomes.	No. of Medium chroms.,	No. of short chroms.,	Total length in mms
<i>Oroxylum indicum</i> $2n = 30$.	16(4-5 mm)	12(2.8-3.4) mm	2 (2.4) mm	108. mm
<i>Millingtonia hortensis</i> $2n = 30$.	10(5.0-3.9) mm	18(3.7-3.2) mm	2(2.5) mm	106.1
<i>Amphilophium Mustisii</i> , $2n = 44$.	12(5.4-4.1) mm	20(3.8-3.0) mm	12(2.8) mm	162.1
<i>Tabebuia guayacan</i> , $2n = 40$.	12(3.6-4.2) mm	20(2.6-3.3) mm	8(2.4) mm	125.2
<i>Tabebuia rosea</i> , $2n = 40$.	14(3.0-3.4) mm	18(2.4-2.8) mm	8(2.2) mm	109.0
<i>Phyllarthron comorense</i> , $2n = 40$.	4(4.0-5.0) mm	24(2.7-3.4) mm	12(2.3) mm	115.7
<i>Pajanelia Rheedii</i> , $2n = 40$.	20(4.0-3.0) mm	18(2.0-2.6) mm	2(1.8) mm	108.4
<i>Tecoma capensis</i> , $2n = 34$.	8(5.7-3.9) mm	18(3.0-3.2) mm	8(2.4) mm	110.5
<i>Tecoma rosea</i> , $2n = 38$.	6(3.8-4.1) mm	22(3.0-3.4) mm	10(2.4) mm	116.4
<i>Tecoma grandiflora</i> , $2n = 38$.	6(6.0-3.8) mm	28(2.6-3.4) mm	4(2.2) mm	114.9
<i>Tecoma radicans</i> , $2n = 40$.	2(4.0 mm)	26(2.8-3.2) mm	12(2.0) mm	111.0

IV. DISCUSSION: (a) Chromosome number In Relation to Taxonomy.

An attempt to classify plant species on the basis of chromosome numbers was first made by Jörgensen (1928). He divided the plant genera into three classes namely, (1) species showing the same chromosome number in a genus; (2) Genera exhibiting a multiple relation with reference to their chromosome numbers and (3) Genera exhibiting aploid chromosome numbers. Tischler (1937) has also made a similar classification of plant genera on a cytological basis. He recognized 4 types which are as follows :—

- (1) 'Those in which all the species have the same chromosome number (*Pinus-type*)'
- (2) 'Those in which the species have multiple or polyploid chromosome numbers (*Chrysanthemum-type*)'
- (3) 'Those in which the numbers are different but not polyploid (i.e., dysploid) (*Carex-type*)'
- (4) 'Those in which the number of chromosomes shows only small variations of 1 or 2 (*Antirrhinum-type*).'

From the point of view of Tischler's classification, it may be seen that most of the genera

and species of the family *Bignoniaceae*, conform to the *Pinus*-type. Not only the different genera exhibit the same chromosome number $2n = 40$, but also the different species belonging to the same genus, as can be seen in the genera *Tabebuia* and *Dolichandrone*. The *Chrysanthemum*-type has been found in a portion of the genus *Bignonia*. The two species, *Bignonia gracilis* and *B. tweediana* ($2n = 80$) exhibit typical polyploidy with reference to those species of *Bignonia* with a diploid complement of $2n = 40$ chromosomes.

The different species of *Tecoma*, like *Tecoma capensis*, *Tecoma Smithii*, *Tecoma rosea* and *Tecoma radicans*, which show a diploid chromosome number, 34, 36, 38 and 40 respectively, can be referred to the *Antirrhinum*-type. Thus on the basis of the data on hand, it may be said that the different sections of the family *Bignoniaceae* can be brought under the *Pinus*-type, *Chrysanthemum*-type and the *Antirrhinum*-type, the first one being very dominant. It is not surprising that all the three types are represented in one and the same family. Tischler (1937) himself has stated that "all the 4 types mentioned may occur in different sections of the same genus."

(b) **Polyploidy** :—Winge (1917) has studied Chromosome numbers in plant species, from a

theoretical point of view and has reached the conclusion, that there is a high frequency of multiples and a low frequency of the primes. This postulation has given a fair clue to the prevalence of polyploidy among the Angiosperms. Since then, considerable number of plant species have been recognized, in which, polyploidy has been found to be a characteristic feature. An examination of the lists of chromosome number in plants compiled by Gaiser (1930) and Tischler (1935,1936,1938) would show that, more than $\frac{1}{2}$ of the genera exhibit this phenomenon. Typical polyploid series are met with in *Chrysanthemum*, *Triticum*, *Solanum*, *Salix*, *Rubus*, *Rosa*, *Senecio* etc.,

Polyploidy is an important factor in the evolution of new species and genera. According to Winge (1924), new types with higher chromosome numbers are evolved, when two species having the same chromosome number, but at the same time having insufficient affinity among the two sets, are crossed either naturally or artificially. There will be asynapsis in the resulting hybrid. If however, duplication of the chromosome sets takes place through longitudinal split, there would result a species, which will be perfectly stable. This species will have twice the number of chromosomes and therefore will be different from either of the parents.

That even Linnean species could be synthesized in the manner cited above, will be evident from the experiments of Müntzing (1930). He crossed two 'good Linnean species'—*Galeopsis pubescens* and *G. speciosa* both having 8 haploid chromosomes. The resultant hybrid was a tetraploid with 16 haploid chromosomes and was identical in every respect, with the old Linnean species, *Galeopsis tetrahit*. Thus, through hybridization, new species could be formed. This is in fact, a view very much advocated by Lotsy (1925). Clausen (1927), has also illustrated a scheme for showing, how new species can be evolved, through increase in the chromosome sets; that is through polyploidy. There are two types of polyploidy—Autopolyploidy and Allopolyploidy.

Autopolyploidy :—Gates (1909), was the first to describe autopolyploidy. He found this in a gigas mutant of *Oenothera Lamarckiana*. Since then, this phenomenon has been reported for a number of plant species. Darlington (1937), has given a list of autopolyploids. In autopolyploids there are found more than two complete sets of identical chromosomes and consequently, a high degree of multivalent formation. From the point of view of speciation, autopolyploidy does not seem to be as important as allopolyploidy. However, Müntzing (1936), has given much importance to autopolv-

ploidy in the evolution of new species. No doubt, there are a few cases where, autopolyploids have not only been successfully established but also have scored over the ordinary diploids.

For instance, Anderson and Sax (1936), have reported the occurrence of an entire group of vigorous autopolyploids in the genus *Tradescantia*. These, unlike the usual autopolyploids, were found to reproduce themselves by seeds. Such cases are indeed, very rare. There is no doubt that of the two phenomenon, allopolyploidy and autopolyploidy, it is the former which plays a more significant part in the evolution of new species.

The chief reason for the failure of the autopolyploids lies in the fact that in these fertility is greatly affected. Owing to the unequal distribution of the chromosomes among the tetrads or pollen grains, most of the autopolyploids are only sterile. They are multiplied chiefly by vegetative means. The *Tradescantia* tribe recorded by Anderson and Sax (1936) is a unique instance.

So far as the investigated species of the Bignoniaceae are concerned, there is no indication as to the existence of any autopolyploids. This inference is drawn from the fact that in the meiosis of the several species, included in

the present study, no multivalents were observed. Further, almost all these species normally reproduced by seeds, although some are propagated through cuttings. Over and above these, the cytological data obtained would warrant the assumption that these are mostly in the nature of allopolyploids.

Allopolyploidy :— There is no dearth of allopolyploids in nature. A number of allopolyploids have also been raised by artificial crossing. The *Raphano-Brassica* obtained by Karpechenko (1928, 1929) is a classical example. The synthesis of the artificial *Galeopsis tetrahit* from *G. pubescens* and *G. speciosa* by Müntzing (1930) is another good example. As a result of crossing *Salix viminalis* ($n=19$) and *S. caprea* ($n=19$) Nilsson (1931), obtained a tetraploid in the F_2 generation which was identical with *S. cinerea*. In all these cases, as a result of hybridization, allopolyploids of a stable type have been obtained. These instances would also bring home the vast potentialities and the wide scope that exist in this method, in the evolution of new species.

The wide occurrence of allopolyploids under natural conditions, is on account of the many advantages that these possess over the ordinary diploids. According to Erlanson (1938), polyploid forms are better fitted to withstand arctic or alpine conditions, while the diploid forms

will simply perish. Navashin (1929) has pointed out that "Through changes in the rate of development, a polyploid individual may acquire the ability of withstanding different climatic conditions and as a consequence, penetrate into new territory." Hagerup (1933) also has stated that "Polyploid forms may be oecologically changed, so as to grow in other climates and formations, where the diploid forms will not thrive." That polyploids are better adapted to alpine conditions than the diploids, has been demonstrated with reference to *Biscutella laevigata* (Manton, 1934) and *Tradescantia* (Anderson and Sax, 1936).

It has been stated already that *Bignonia gracilis* and *B. Tweediana* are in the nature of tetraploids with reference to those species of *Bignonia* which exhibit a diploid chromosome number of 40 chromosomes. To what extent this tendency prevails in this genus cannot be definitely stated at present; for the genus, *Bignonia* is a very big one and comprises about 500 species (Hooker and Jackson, 1895) of which, chromosome number has been determined only for 9. Besides such instances where the polyploid nature of the species is indicated, there are also several other species especially, the 40-chromosomed ones, where the same tendency has been inferred. The meiotic studies in forms like *Crescentia Cujete*, *Tabebuia pentaphylla*,

Parmentiera cerifera, *Millingtonia hortensis*, *Tecoma capensis*, and *Tecoma stans* have revealed the presence of secondary association.

According to Lawrence (1931), secondary association is 'intimately connected with allopolyploidy.' So, on the basis of the occurrence of secondary association, the above mentioned species can be regarded as allopolyploids. The study of secondary association in some of these has thrown some light regarding the basic number. The maximum association found in *Crescentia Cujete*, *Tabebuia pentaphylla* and *Parmentiera cerifera* is 10 and the number of groupings was found to be also 10. This would mean that these 40-chromosomed members are allotetraploids derived from 10-chromosomed ancestors.

The allotetraploid nature of these species would also receive support from other pieces of evidence such as, absence of multivalent formation, attachment of two bivalents to the nucleolus during diakinesis, as in *Kigelia pinnata*, the occurrence of 2 nucleoli at telophase I as in the PMC., of *Crescentia Cujete*, the presence of four nucleoli in the somatic telophase and the attachment of 4 prochromosomes to the nucleolus at the resting condition as observed in *Oroxylum indicum*. The significance of these has been discussed already, as well as

the possible method of origin of the 40-chromosomed species from a 10-chromosomed ancestor, in the light of all available cytological data.

(c) **Aneuploidy**:—The chromosome numbers observed in aneuploid species do not bear a multiple relationship as in polyploid forms. A characteristic feature of aneuploids seems to be, that some odd chromosomes are duplicated or eliminated. This phenomenon on the whole is less understood than polyploidy. Aneuploidy seems to be a rarity among the wild plants. It is mostly observed among the cultivated species. The various strains of *Zea Mays*, *Triticum*, *Datura*, *Nicotiana* and *Primula* under cultivation, afford instances of this kind. Hollingshead and Babcock (1930) found in *Crepis syriaca* a regular aneuploid series such as $2n = 10, 11, 12, 13$ etc., *Crepis tectorum* also exhibited aneuploid numbers though only to a very small extent (Navashin, 1926).

The infrequent occurrence of aneuploidy has been pointed out already. According to Manton (1932), the various genera of the family *Cruciferae* show an aneuploid relationship. A similar relationship among the genera of the family *Leguminosae* has been reported by Jacob (1940). From these and several other instances, it would appear that aneuploidy is

more frequent among the related genera than among related species.

In the family Bignoniaceae, however, a unique instance of regular aneuploid series has been observed among the few investigated species of *Tecoma*. These are, *Tecoma capensis* ($2n = 34$, $n = 17$), *Tecoma Smithii* ($2n = 36$, $n = 18$), *Tecoma rosea* ($2n = 38$), *Tecoma australis* ($2n = 38$), *Tecoma grandiflora* ($2n = 38$) *Tecoma chrysantha* ($2n = 38$) *Tecoma Tagliabuana* ($2n = 40$) *Tecoma radicans* ($2n = 40$) and *Tecoma stans* ($2n = 40$, $n = 20$).

These different chromosome numbers, 34, 36, 38 and 40 could have been resulted either through duplication, or through elimination. If it is due to duplication then, the 34-chromosomed member would be the starting point. If it is a case of elimination, the same series could also result from the base, 40. Starting from the 40-chromosomed type, numbers like 38, 36 and 34 may be obtained through end to end fusion of some of the chromosomes.

Such an end to end fusion of chromosomes has been reported in the various genera of the family *Malvaceae* (Davie, 1933). According to Darlington (1937) *Fritillaria ruthenica* and *Stenobothrus* have been derived from ancestral forms by the same process. *Cardamine pratensis* ($n = 15$) has been interpreted as a tetraploid

with an original basic number of 8, where according to Lawrence, (1931), two of the chromosomes have undergone fusion. Thus there are three alternative methods by which the numbers 34, 36, 38 and 40 could be obtained, namely, duplication of some of the chromosomes, elimination and fusion of the chromosomes.

Of the three methods, duplication is much simpler than the rest, and it is likely, that it is by this process the various chromosome numbers have been produced in the different species of *Tecoma*. This inference is based on the data gained through the analysis of the chromosomes, with reference to their length, in the different species. The following is an extract from table 2.

Species	Long chromo- somes	Medium	Short	Total length
<i>Tecoma capensis</i> ($2n = 34$)	8	18	8	110.5 mm
<i>Tecoma grandiflora</i> ($2n = 38$)	6	28	4	114.9 „
<i>Tecoma rosea</i> , ($2n = 38$)	6	22	10	116.4 „
<i>Tecoma radicans</i> ($2n = 40$)	2	26	12	111.0 „

A striking feature which can be made out from the figures given above, is that the total chromosome length in the different species with chromosome numbers, 34, 38 and 40, is more or less the same, provided an error of 5% is allowed. (This error will not be too much considering the magnification at which the drawings

have been made and the further error which is unavoidable in measuring the chromosome length.) Another interesting feature is that there is a reduction in the number of long chromosomes, as one proceeds from the 34-chromosomed type to the one exhibiting 40 somatic chromosomes. Thus *Tecoma capensis* ($2n = 34$) has 8 long chromosomes, while those in which the prevailing numbers are $2n = 38$ and $2n = 40$, exhibit only 6 and 2 long chromosomes respectively.

Corresponding to this decrease in the number of long chromosomes, there is an increase in the number of short chromosomes. Another significant feature that can be made out by reference to table 2 is, that in the different species of *Tecoma* considered above, the length of two short chromosomes or the length of a short chromosome and a medium one is approximately equal to that of a long chromosome.

That the total length of the chromosomes in the different aneuploid species of *Tecoma* remains the same, will lead to the inference, that these numbers could not have been resulted due to elimination of chromosomes. This reduces the choice to two namely, end to end fusion and duplication of the chromosomes. Since the length of any two short chromosomes or that of a short and a medium one corresponds to the

length of a long chromosome, it may be assumed that fusion of the short and medium chromosomes might have taken place. If such a fusion had taken place, the long chromosomes thus formed, ought to exhibit secondary constrictions, corresponding to the two attachment constrictions of the two involved chromosomes, since the centromere is the most stable part of the chromosome. According to Davie (1933), the long chromosomes formed as a result of fusion in *Lavatera olbia*, show secondary constriction. The long chromosomes in none of the species of *Tecoma* examined, revealed the presence of secondary constriction. The absence of secondary constriction in the somatic chromosomes of the *Tecoma* species examined would therefore rule out end to end fusion also. There now remains only the process of duplication by which the aneuploid numbers could have resulted.

Duplication of chromosomes, according to Rosenberg (1918), would take place mostly through non-disjunction. In the somatic cells polysomy or duplication of the chromosomes may be caused by longitudinal fission. So far as the few *Tecoma* species are concerned, duplication of the chromosomes could not have been due to non-disjunction; for, in the two species of *Tecoma*, (*Tecoma stans* and *Tecoma canensis*), where meiosis was observed

there was found no indication of non-disjunction. If there was any, the second metaphase plates would not have exhibited an equal distribution of chromosomes.

The possibility of duplication of chromosomes in the somatic cells due to longitudinal fission, was considered next. If this had taken place, the number of long chromosomes in the different species exhibiting the aneuploid series, ought to have remained the same. It has already been pointed out that this is not the case and that there is a decrease in the number of long chromosomes, as one examines the 34-40 series.

From what has been said above, it will be evident that the aneuploid numbers in the *Tecoma* species have been produced neither due to non-disjunction nor due to longitudinal fission of the somatic chromosomes. The fact that there is a regular decrease in the number of long chromosomes in the 34-40 series, would suggest another possibility. It is quite likely that instead of longitudinal fission of the somatic chromosomes, there might have been transverse septation. The following points are in favour of this view:—

- (1) The presence of a large number of somatic chromosomes exhibiting median or submedian constriction. (This

would facilitate septation in the region of the centromere).

- (2) The length of two short chromosomes or that of a short and a medium one is equal to that of a long chromosome. (This gives a clue, that a few of the long chromosomes are likely to have been divided into two short chromosomes or into a short and a medium one depending upon the position of the attachment constriction; if it is a chromosome with median constriction, that was affected then, there would result two short chromosomes of approximately equal length. A chromosome with submedian constriction, on the other hand, would result in a short and a medium chromosome.)
- (3) There is a progressive increase in the number of short chromosomes in the 34-40 series. (In the $2n=34$ species, there are only 8 short chromosomes; in the $2n=38$, there are 10 and in the $2n=40$ species there are 12 short chromosomes).
- (4) The absence of secondary constriction in the somatic chromosomes of the different *Tecoma* species exhibiting aneuploid numbers.

From all these, it is concluded that transverse septation of the somatic chromosomes is likely to have resulted in the production of the aneuploid series, so well exhibited in the few investigated *Tecoma* species.

Transverse septation of chromosomes or fragmentation, though of infrequent occurrence, has played an important part, in the production of forms with supernumerary chromosomes. For instance, Metz (1916) interpreted the increase in the number of chromosomes in a few species of *Drosophila*, as being due to the breaking up of the V-shaped chromosome into two I-shaped ones, representing the arms of the V. The normal chromosome number in *Zea Mays* is $n=10$. But Kuwada (1919), isolated a few strains of *Zea Mays* having supernumerary chromosomes and he regarded the extra chromosomes as having been originated by a process of fragmentation; but Randolph (1927), does not agree with Kuwada, regarding the manner in which, these extra chromosomes have arisen. Morgan and others (1922) also have provided sufficient cytological and genetical evidence, to warrant the statement, that transverse septation and end to end fusion of chromosomes are much more common, than has been considered hitherto. In *Oenothera Scintillans*, the somatic cells exhibit chromosome numbers ranging from 15-21. This varia

tion is believed to be due to a process of transverse division of the chromosomes (Hance, 1918). In *Hyacinthus* (de Mol 1921) it was found that the sum of the length of the medium and short chromosomes, is equal to the length of the long chromosomes and therefore, the possibility of transverse division of the chromosomes has been suggested. Belling (1925) regarded the 16-chromosomed karyotype of *Secale cereale*, as having been evolved from the 14-chromosomed type by fragmentation. The same suggestion has also been made by Emme (1928) and Levitsky (1929) to explain the occurrence of species of *Secale cereale*, exhibiting more than 14 chromosomes in their somatic cells.

An unmistakable piece of evidence of fragmentation is provided by the material *Carex* (Heilborn, 1924). A few of these species, show long, medium and short chromosomes. Heilborn (1924) made critical measurements of the chromosome complements of the different species of *Carex* and found that the medium and short chromosomes were the products of fragmentation of the long chromosomes. The genus *Tulipa* also (Newton, 1926) provides an instance of this kind. The prevailing number in this genus is $n = 12$. However, *Tulipa galatica* shows $n = 16$ and this number has arisen by a process of segmentation from the 12-chromosomed *Tulipa* species (Newton, 1926).

The two phenotypes of *Scirpus pulsatris* ($n=8$ and $n=19$), afford yet another similar example. According to Håkansson (1927, 1929), the 19-genomed type is a tetraploid form in which some of the chromosomes have undergone segmentation.

Transverse segmentation has also been reported in a few species of *Allium*. Most species of *Allium*, exhibit $2n=16$ chromosomes. However, there are a few like, *A. Karataviense* and *A. triquetrum*, having $2n=18$ chromosomes. Levan (1932) has made a detailed investigation of these species and arrived at the conclusion that the 18-chromosomed *Allium* species, have arisen from the 16-chromosomed *Allium* species by a process of fragmentation. He also actually observed in *A. validum*, one of the long chromosomes (with submedian constriction) in the process of transverse segmentation.

It will thus appear, that septation of the chromosomes has been the cause, for the numerical increase in the chromosome number, of the different species of *Tecoma* included in the present study.

When a chromosome divides into two bits by transverse division, only one of these is likely to carry the centromere. According to Newton (1926), if a fragment is to have any

then it must develop a kinetochore. Darlington (1929) also has emphasized the same aspect, namely, that an originated fragment can survive only if it developed an insertion constriction. This he has referred to as the 'mitotic condition'. Acentric fragments, on the other hand, will soon disappear and may not have much value (Darlington, 1937).

However, it is quite possible that such fragments, even though they may not have an attachment constriction at the time of their formation, may develop one, if they happen to persist for a few generations. In diploid *Urginea indica*, (Raghavan and Venkatasubban, 1940b), there were found, 1, 2, 3 or even 4 fragments, besides the usual number of chromosomes namely 20, in the root tip cells. These fragments did not reveal the presence of any constriction. However, during diakinesis, they were found to conjugate and behave just like very small chromosomes. Persistence of fragments, has also been noted in *Scilla indica* (Raghavan and Venkatasubban, 1939). In this species, the fragments during meiosis were actually seen to divide and migrate to the poles, although the division and migration were somewhat erratic. These examples would go to show that acentric fragments need not necessarily perish but can survive and form new centromeres.

(d) **Speciation** :—Several species and genera of the family Bignoniaceæ were examined with a view to find out how species have been formed in this family. In the formation of species, there may be a numerical variation in the chromosomes. Difference in the size of the chromosomes may also be responsible for species-formation. This is best seen in the two clones of *Fritillaria*, where the bigger clone had bigger chromosomes, while, the smaller one exhibited only smaller chromosomes (Darlington, 1937). Structural changes like, segmental interchange, duplication, inversion and deletion of a portion of the chromosome are also likely to be involved in the process of species-formation.

Among the members of this family, variation in chromosome numbers has been found only on a small scale. While the majority of the investigated species exhibited a diploid chromosome number of $2n=40$, a few like *Bignonia gracilis*, *B. Tweediana*, *Tecoma capensis*, *Tecoma Smithii*, *Tecoma rosea*, *Tecoma stans* etc., revealed variations in chromosome numbers, ranging from 34 to 80. The different species, however, did not exhibit any disparity in the size of their somatic chromosomes. It may also be mentioned incidentally, that the drawings illustrating the somatic complements of *Incarvillea Delavayi* and *I. grandiflora*

(Sugiura, 1936) appear to be quite unlike the chromosomes figured and studied in the present paper. The chromosomes of *Incarvillea Delavayi* and *I. grandiflora* are considerably bigger than the rest of the species.

Therefore it is likely, that chromosome size might have also played a small part in the formation of species in this family. It may be assumed that those species in which there is neither difference in the number nor in the size of the chromosomes, are likely to have been subjected to structural changes in their chromosomes. There is no positive evidence such as the formation of rings, for this conjecture. However, it may be remembered that structural changes can take place without the manifestation of rings. Clarke and Anderson (1935) have recorded a similar instance, namely, reciprocal translocation without any ring formation, in a species of Maize. It has been pointed out already, that on account of the very small size of the bivalents, rings even though they might have been formed, are not likely to be made out. The association of more than two bivalents in the first metaphase and in subsequent stages, of meiosis, in a number of species, has been interpreted as providing some evidence of structural changes in the chromosomes concerned. This has been discussed at some length in Part II, under secondary association.

The preponderance of rod-bivalents to ring-bivalents, exhibiting only one terminal chiasma in species like, *Tabebuia pentaphylla*, *Millingtonia hortensis*, *Crescentia Cujete* and *Kigelia pinnata* would point out to the feeble affinity between the conjugating chromosomes. This feeble affinity might have been caused by structural changes in the chromosomes. The structural changes in most of these chromosomes, might have been due to gene mutation also. Therefore it is probable that in the formation of species in this family the following processes have been involved:—(a) Alteration in Chromosome number, (b) Alteration in Chromosome size, (c) structural changes and (d) gene-mutation.

(e) **Phylogenetic considerations** :—According to Bentham and Hooker's system of classification, the family *Bignoniaceae* has been divided into 4 tribes—*Bignoniaceae*, *Tecomeae*, *Jacarandaceae* and *Crescentieae*. In Engler and Prantl's system, there are 5 tribes instead of 4. Two of these tribes namely, *Eccremocarpeae* and *Tourreantieae* are monogeneric, each containing the single genus, *Eccremocarpus* and *Tourreantia* respectively. These genera could not be included in the present study since materials of these could not be procured. Omitting these for the present, there are the three tribes, *Bignoniaceae* *Tecomeae* and *Crescentieae*.

A comparison of the two systems would show that, there is nothing corresponding to the tribe *Jacarandae* in the system by Engler and Prantl. The tribe *Jacarandae* as given in *Genera Plantarum* (Bentham and Hooker Vol. II 1873-76) includes the following genera: *Eccremocarpus*, *Parmentiera*, *Colea*, *Digomphia* and *Jacaranda*. Engler and Prantl have included the two genera, *Colea* and *Parmen-tiera*, under the tribe *Crescentieae*, *Jacaranda* and *Digomphia* are included under the tribe *Tecomeae*, while the genus *Eccremocarpus* was elevated to the rank of an independent tribe.

From the point of view of chromosome numbers, *Jacaranda* can as well be included under the tribe *Tecomeae* as has been done by Engler and Prantl; for, there are other members in this tribe like *Campsis grandiflora* and *Tecoma Smithii*, exhibiting the same number $2n=36$. The inclusion of the genus *Parmen-tiera* in the tribe *Crescentieae* as has been done by Engler and Prantl, is also supported from the point of view of chromosome numbers. The few species belonging to this tribe, which have been investigated, revealed the diploid number $2n=40$. The inclusion of *Parmentiera* under the tribe *Crescentieae* is also supported by secondary association studies since, in both *Parmentiera cerifera* and *Crescentia Cujete*,

the maximum association and basic number have been found to be 10.

From purely cytological considerations, an attempt has been made to assemble the various genera and species phylogenetically. No doubt, chromosome numbers alone are inadequate, in such an attempt, as it is not absolutely diagnostic. A further handicap is imposed on account of the fact that chromosome numbers are available at present only for a limited number of species in this family. Nevertheless a tentative scheme has been drawn and only further data regarding the chromosome numbers of other uninvestigated species, together with cytological details such as secondary association etc., would help to confirm or modify the present scheme. In drawing up this scheme, in addition to chromosome numbers, cytological evidence gained from secondary association, nucleolar behaviour etc., have also been employed.

Secondary association has been observed in several species, but was studied in detail only in a few like *Crescentia Cujete*, *Tabebuia pentaphylla* and *Millingtonia hortensis*. In *Crescentia Cujete* and *Tabebuia pentaphylla*, the maximum association was found to be 10 and the same was also found to be the base number. From this, it has been concluded that the 40-chromosomed members are likely to be

in the nature of allotetraploids, derived from the basic number 10. The tetraploid nature of some of these has been inferred not only from the occurrence of secondary association, but also from the nucleolar behaviour in species like *Kigelia pinnata*, *Crescentia Cujete* etc., These observations together with the fact, that the majority of the species investigated so far, show a diploid chromosome number of $2n=40$, would support the conclusion that 10 is likely to be the primary basic number for this family.

The same considerations in *Millingtonia hortensis*, revealed the possibility of another basic number namely 8. If the lowest number met within this family is also taken into consideration, the presence of yet another basic number 9 is revealed. This is found in the two species of *Incarvillea*, for which chromosome number is now available. Thus, there is a certain amount of evidence to show that the family may have the basic numbers, 10, 9 and 8. If 10 is regarded as the primary basic number—an assumption made on the basis of the greater frequency of the 40-chromosomed species within the family, then, 9 and 8 are to be considered as secondary basic numbers. It is likely that by the fusion of two chromosomes, the basic number 9 could be obtained from 10; by the fusion of 4 chromosomes,

Keeping the various genera under the respective tribes as indicated in the system of Engler and Prantl, a possible course of evolution of the various investigated species is suggested with reference to the basic numbers 8, 9 and 10. Almost all the 40-chromosomed species are traced back to the basic number 10. The 10-chromosomed stock may be imagined to have given a branch one in the direction of the tribe, *Crescentieae*, another in the direction of the tribe *Tecomeae* and a third one towards the tribe *Bignoniaceae*. For in all these tribes there are several 40-chromosomed members and these species might have been evolved from 10-chromosomed ancestor through amphidiploidy. The 40-chromosomed stock in the genus *Bignonia*, might have given rise to other numbers like 44, 50 and 80, seen in the various members of the tribe *Bignoneae*. Two species of the same tribe *Bignoneae* namely, *Millingtonia hortensis* and *Oroxylum indicum* exhibit a diploid chromosome number of $2n=30$. In the former the basic number has been found to be 8. A tetraploid could have arisen from this basic number (8), through amphidiploidy and if two of the chromosomes are eliminated the result will be a 30-chromosomed species. The tetraploid nature of *Oroxylum indicum* is, seen from the fact that in the somatic mitosis, 4 nucleoli were seen during early telophase

(text fig. 42). In addition to this, 4 prochromosomes were also found attached to the nucleolus at the resting stage of the somatic nucleus (text fig. 35). The tetraploid nature of *Millingtonia hortensis* has been inferred from the occurrence of secondary association. Therefore both *Millingtonia hortensis* and *Oroxylum indicum* may be regarded as allotetraploids from a 8-chromosomed stock. Owing to the loss of 2 chromosomes these show only 30 instead of 32 chromosomes as would be required of the allotetraploids. Thus the members of the tribe *Bignoniaceae* are traced to two different stocks namely the one with 10 chromosomes and the other with 8 chromosomes.

In the tribe *Tecomeae*, the occurrence of the following chromosome numbers in the different species can be explained as follows:— It has been referred to already that the numbers 34, 36, 38 and 40 might have been produced by transverse septation of a few of the long chromosomes, starting from the number 34. The number 34 which is the lowest so far recorded for the genus *Tecoma*, can be derived, if it is assumed that a cross had taken place between the forms with 8 and 9 chromosomes, just in the same way, the artificial *Galeopsis tetrahit* was synthesized out of *G. pubescens* and *G. speciosa* (Müntzing 1930). The difference in the chromosome numbers would not

stand in the way of a successful crossing as has been demonstrated by U (1935) in the cross between *Brassica campestris* ($n=10$) $\times$ *B. oleracea* ($n=9$). As a result of this cross *Brassica napus* ($n=19$) was formed with a new basic number. By a similar process the number $n=17$ observed in a few species of *Tecoma*, might have been evolved from the basic numbers 8 and 9. The two species of *Incarvillea* exhibiting $2n=18$, can be derived directly from the basic number 9.

Regarding those which exhibit the number $2n=36$, these can be derived directly from the basic number 9. But as has been pointed out before, the 9—chromosomed type—*Incarvillea* species exhibit chromosomes which are quite different from those that have been reported in the present investigation. The chromosomes as drawn by Sugiura (1936) are very much bigger as compared to the various species now investigated. In other words, the species reported in this paper are mostly small-chromosomed ones and *Jacaranda mimosaefolia* showing $2n=36$ is no exception to this. If *Jacaranda mimosaefolia* has had its ancestry in the 9—chromosomed type then it should have bigger chromosomes as are found in the two *Incarvillea* species. But it is not so. Therefore it is likely that its ancestry lies in those forms having only small chromosomes. The 8-chro-

mosomed type, is likely to be one of small chromosomes so far as could be judged from the chromosome-size in *Oroxylum indicum* and *Millingtonia hortensis*. So also the 10-chromosomed type as can be seen in all those species with 40 chromosomes. If a cross had taken place between the 8 and the 10 types, the result would be a form with $n=18$ and the chromosomes will also be small as seen in *Jacaranda mimosaefolia* and allied species.

The prevalence of a common chromosome number of approximately the same size in the different species of this family, would afford wide scope both for intergeneric and interspecific crossing. That such crosses are quite feasible is borne out by the following examples:—*Tecoma Smithii* has been found to be a hybrid between *Tecoma mollis* and *Tecomaria capensis*. This hybrid (*Tecoma Smithii*), is said to have been raised in Australia, by Godwin Smith (Bailey, 1935). Again, *Catalpa hybrida* provides another example to show that interspecific hybridization is quite possible and can be effected successfully among the members of this family. According to Bailey (1935), *Catalpa hybrida* has had its parentage in *Catalpa bignonioides* and *Catalpa ovata*. Hybrids have been produced by crossing *Campsis radicans* and *Campsis chinensis* by Sax (1933).

Lastly, it may be pointed out that the presence of 3 different basic numbers among the members of one and the same family, need not be incongruous. For there are several families exhibiting more than one basic number. Sometimes even a single genus may reveal the presence of several basic numbers as has been pointed out by Babcock and Cameron (1934), for *Crepis*.

The origin of the different species with different chromosome numbers, from the basic numbers, 8, 9 and 10 is illustrated in a diagrammatic manner. In the illustration, the basic numbers 8 and 10 are followed by an interrogative mark, since these are merely hypothetical numbers at present. Only further investigation would show the validity of these assumptions.

V. SUMMARY

Chromosome numbers for 35 species spread over 16 genera have been recorded for the first time. The numbers have been found to range from $2n = 18$ to $2n = 80$, $2n = 40$ being the number most often met with.

The chromosomes in the different species, show submedian or subterminal constriction, besides being small and almost alike.

Prochromosomes have been met with in most of the species investigated and their beha-

viour in mitosis has been followed in *Oroxylum indicum*.

Regular aneuploid species exhibiting the numbers, 34, 36, 38 and 40 in the genus *Tecoma* have been observed.

Evidence from secondary association studies, nucleolar behaviour and chromosome numbers go to show that most of the 40-chromosomed species are likely to be in the nature of allotetraploids derived from the basic number 10.

Polyploidy has been found in the genus *Bignonia*.

The two species, *Millingtonia hortensis*, and *Oroxylum indicum* are regarded as hypo-allotetraploids with $4x-2$ chromosomes from the basic number 8.

Three basic numbers, 8, 9 and 10 have been suggested for the family and the number 10 is regarded as primary.

Hybridization, amphidiploidy, change in chromosome number, structural changes and gene mutation seem to have played a part in speciation in this family.

A tentative scheme has been put forward on the basis of all available cytological data to show the possible course of evolution of the different species from the basic numbers, 8, 9 and 10.

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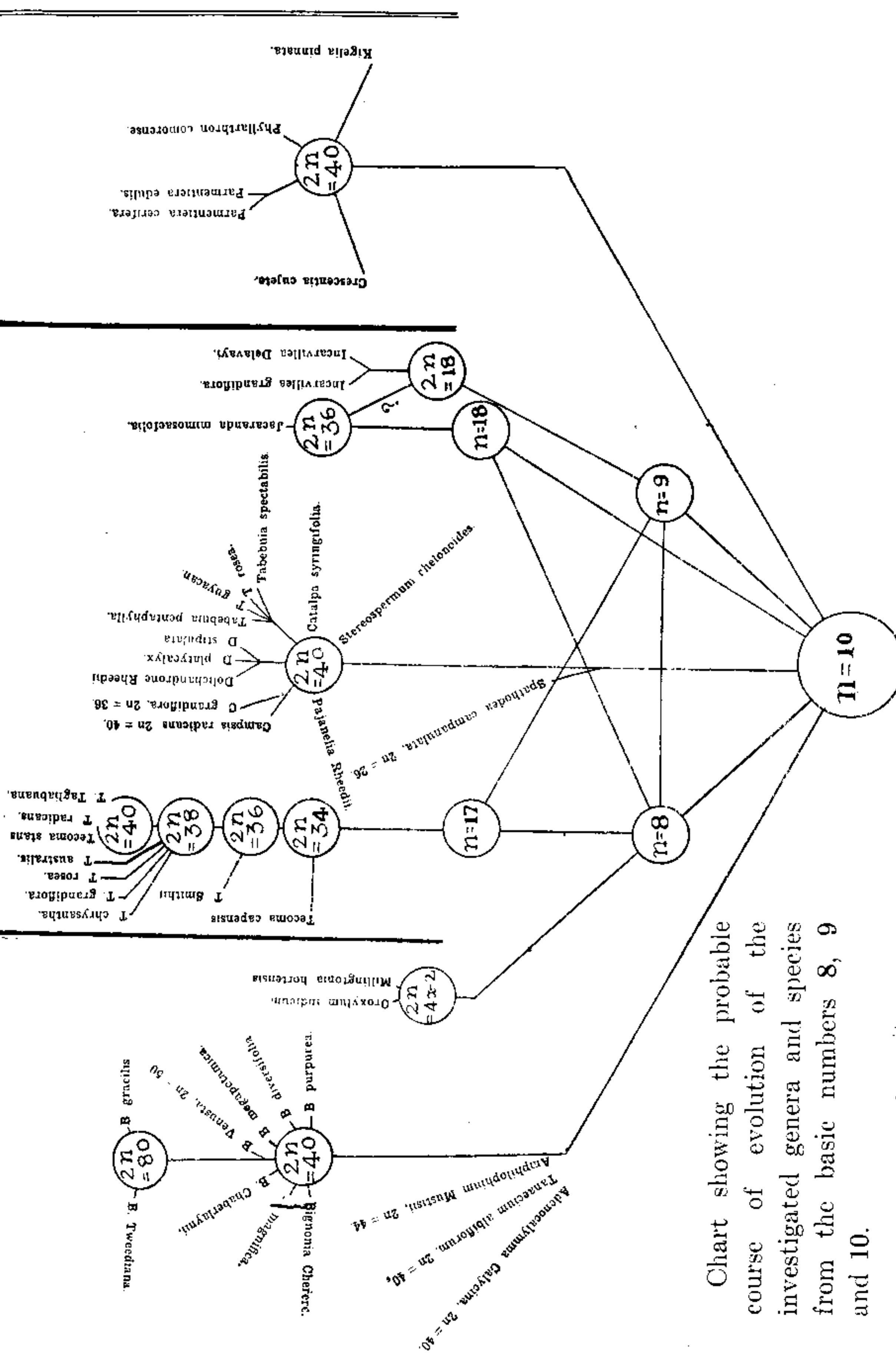
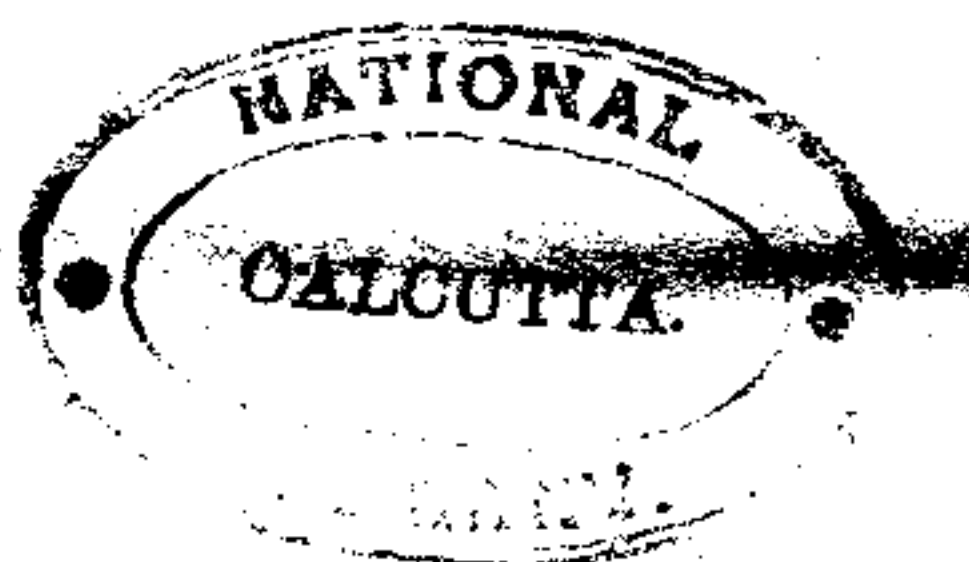


Chart showing the probable course of evolution of the investigated genera and species from the basic numbers 8, 9 and 10.



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